WEST Search History

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DATE: Wednesday, February 28, 2007

Hide?	Set Name	Query	Hit Count
		DB=PGPB,USPT,USOC,EPAB,JPAB,DWPI,TDBD; PLUR=YES; OP=OR	
☐	L1	((ammonia.ti.) and $sensor.ti.)	299
☐	L2	(((MICHLERS | MICHLERKETONE | MICHLERIS | MICHLERE | MICHLER | MICHLERI | MICHLERSKCTONE | MICHLERSKETONE)!) or ((MICHLERS | MICHLERKETONE | MICHLERIS | MICHLERE | MICHLER | MICHLERI | MICHLERSKCTONE | MICHLERSKETONE)!) or ((MICHLERS-KCTONE | MICHLER8)!) or ((MICHLER-KETONE)!) or ((MICHLER-KETONE)!) or ((MICHLER-KETONE | MICHLER-THIOKETONE)!) or ((MICHLER-KETONE | MICHLER/S)!) or ((MICHLER-KETONE | PARA-ROSANILINE | PARA-ROSANILINE-MONOSULPHONIC | PARA-ROSANILIN | PARA-ROSANIL | PARA-ROSANALINE | PARA-ROSALINE | PARA-ROSAIIILINE | PARA-ROSANILNE | PARA-ROSANILIN-DERIVATIVE | PARA-ROSANININE | PARA-ROSANI-LINE | PARA-ROSIIIILIN)!) or ((MICHLER-KETONE | PARA-ROSANILINE | PARA-ROSANILINE-MONOSULPHONIC | PARA-ROSANILIN | PARA-ROSANIL | PARA-ROSANALINE | PARA-ROSALINE | PARA-ROSAIIILINE | PARA-ROSANILNE | PARA-ROSANILIN-DERIVATIVE | PARA-ROSANININE | PARA-ROSANI-LINE | PARA-ROSIIIILIN)!) or ((MICHLER-KETONE | PARA-ROSANILINE | PARA-ROSANILINE-MONOSULPHONIC | PARA-ROSANILIN | PARA-ROSANIL | PARA-ROSANALINE | PARA-ROSALINE | PARA-ROSAIIILINE | PARA-ROSANILNE | PARA-ROSANILIN-DERIVATIVE | PARA-ROSANININE | PARA-ROSANI-LINE | PARA-ROSIIIILIN)!) or ((MICHLER-KETONE | PARAROSANILINE | PARAROSANILINEHYDROCHLORIDE | PARAROSANILINES | PARAROSANILINESULPHONIC | PARAROSANILINE-HYDROGEN | PARAROSANILINE-ETHANOL | PARAROSANILIN | PARAROSANILIIIE | PARAROSANILIDE | PARAROSANILI | PARAROSANILANE | PARAROSANILE | PARAROSANIL | PARAROSANIIINE | PARAROSANIHNE)!) or ((MICHLER-KETONE | ALPHA-NAPHTHOLAZOBENZENE | ALPHA-NAPHTHOLBENZEIN | ALPHA-NAPHTHOLBE | ALPHA-NAPHTHOLBENZALDEHYDEA | ALPHA-NAPHTHOLBENZENE | ALPHA-NAPHTHOLBENZIN | ALPHA-NAPHTHOLBENZOATE | ALPHA-NAPHTHOLBENZOIC | ALPHA-NAPHTHOLBENZYL)!) or ((MICHLER-KETONE | ALPHANAPHTHOL-BENZEIN)!) or ((MICHLER-KETONE | ALPHA-NAPHTHOL-BENZEIN | ALPHA-NAPHTHOL-BENZENE | ALPHA-NAPHTHOL-BENZOIN | ALPHA-NAPHTHOL-BENZEIIA | ALPHA-NAPHTHOL-BENZALDEHYDE-A)!) or ((MICHLER-KETONE | NAPHTHO-CHROME | NAPHTHOCHROME | NAPHTHOCHROM)!))	6351
☐	L3	l1 and l2	0

		DB=TDBD,DWPI,JPAB,EPAB,USOC,USPT,PGPB; PLUR=YES; OP=OR	
☐	L4	(MICHLERSHYDROL | MICHLERSHYDROL260 | MICHLERSHYDROL-260 | MICHLERS | MICHLERS-KCTONE | MICHLERSKETONE | MICHLERSKCTONE | MICHLER8)!	189
		DB=PGPB,USPT,USOC,EPAB,JPAB,DWPI,TDBD; PLUR=YES; OP=OR	
☐	L5	ammonia! near5 $sensor	878
☐	L6	L5 and (l2 or l4)	0
☐	L7	ammonia!	324490
☐	L8	L7 and (l2 or l4)	861
☐	L9	L8 and breath$	10
☐	L10	(l2 or l4).clm. same (devic$ or $device or apparatus or $apparatus).clm.	11
☐	L11	L10 not l9	9
☐	L12	l7 same balloon	269
☐	L13	L12.clm.	6
☐	L14	l12 and l5	2

END OF SEARCH HISTORY

DOCUMENT-IDENTIFIER: US 20020182658 A1
TITLE: Sensor device and methods for manufacture

CLAIMS:

21. The device of claim 1, wherein said quenching dye is selected from the group consisting of an Alkali Blue, Safranin, and Pararosaniline.

Fricker R, Doptis P, Hornak M, Li C. "Development of and optimized ammonia assay for the Kodak Ektachem Analyzer." Clinical Chemistry 1990; 36 (6): 1072

Queres, JC. "Hyperammonemia and Helicobacter pylori." The Lancet 1995; 346-713.

Lahdesmaki, I. Lewenstam, A, et al. "A Propyrrole-based Amperometric Ammonia Sensor." Talanta 1996; 43: 125-134.

Ratnaike RN, Buttery JE, Hoffman S. "Blood Ammonia Measurement Using a Simple Reflectometer." Journal of Clinical Chemistry and Clinical Biochemistry 1984; 22(1): 105-108.

1. 20060222675. 28 Mar 06. 05 Oct 06. Personal care compositions with color changing indicator. Sabnis; Ram W., et al. 424/405; A01N25/00 20060101

☐ 2. 20060222601. 28 Mar 06. 05 Oct 06. Oral care compositions with color changing indicator. Sabnis; Ram W., et al. 424/49; A61K8/368 20060101 A61K8/46 20060101

☐ 3. 20060173433. 01 Feb 05. 03 Aug 06. Absorbent articles comprising polyamine-coated superabsorbent polymers. Laumer; Jason Matthew, et al. 604/372; 604/368 A61F13/15 20060101

☐ 4. 20060173432. 01 Feb 05. 03 Aug 06. Absorbent articles comprising polyamine-coated superabsorbent polymers. Laumer; Jason Matthew, et al. 604/372; 604/368 A61F13/15 20060101

☐ 5. 20060173431. 01 Feb 05. 03 Aug 06. Absorbent articles comprising polyamine-coated superabsorbent polymers. Laumer; Jason Matthew, et al. 604/372; A61F13/15 20060101

☐ 6. 20050112085. 16 Oct 03. 26 May 05. Odor controlling article including a visual indicating device for monitoring odor absorption. MacDonald, John Gavin, et al. 424/76.1; A61L009/01.

☐ 7. 20050085739. 16 Oct 03. 21 Apr 05. Visual indicating device for bad breath. MacDonald, John Gavin, et al. 600/530; A61B005/08.

☐ 8. 20050084977. 16 Oct 03. 21 Apr 05. Method and device for detecting ammonia odors and helicobacter pylori urease infection. Boga, RameshBabu, et al. 436/113; G01N033/53 G01N033/00.

☐ 9. 20030059471. 29 Nov 01. 27 Mar 03. Oral delivery formulation. Compton, Bruce Jon, et al. 424/489; A61K009/14.

☐ 10. 4790857. 20 Nov 86; 13 Dec 88. Gaseous contaminant dosimeter with diffusive material for regulating mass uptake. Miksch; Robert R.. 95/45; 422/101 422/61 422/86 422/88 436/178 436/902 73/863.21 73/864.51 96/11 96/417. B01D053/22 .

Color change dye fluid

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Term	Documents
BREATH$	0
BREATH	35972
BREATHA	18
BREATHAB	9
BREATHABEL	1
BREATHABFE	2
BREATHABFLITY	1
BREATHABIE	6
BREATHABIETY	2
BREATHABIHTY	1
BREATHABIIITY	1
(L8 AND	

BREATH$).PGPB,USPT,USOC,EPAB,JPAB,DWPI;TDBD.	10

There are more results than shown above. Click here to view the entire set.

Prev Page Next Page Go to Doc#

Search Notes

Include all US Patents in search.

US007087265B1

(12) **United States Patent**
Netsch

(10) **Patent No.: US 7,087,265 B1**
(45) **Date of Patent: Aug. 8, 2006**

(54) **IMAGE DEVELOPING DEVICE**

(76) Inventor: **Bryan A. Netsch**, 444 Remington Pt., Highland Village, TX (US) 75077

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

(21) Appl. No.: **10/906,244**

(22) Filed: **Feb. 10, 2005**

(51) **Int. Cl.**
B41M 5/20 (2006.01)

(52) **U.S. Cl.** **427/145**; 101/483; 427/1; 427/150; 427/152

(58) **Field of Classification Search** ..... None
See application file for complete search history.

(56) **References Cited**

U.S. PATENT DOCUMENTS

4,321,093 A	*	3/1982	Seitz	523/161
4,865,938 A		9/1989	Sakai et al.	430/138
4,943,089 A	*	7/1990	Reardon	283/81
6,114,281 A	*	9/2000	Belding et al.	503/201
6,162,485 A		12/2000	Chang	427/1
2003/0236165 A1		12/2003	Peebles et al.	503/215

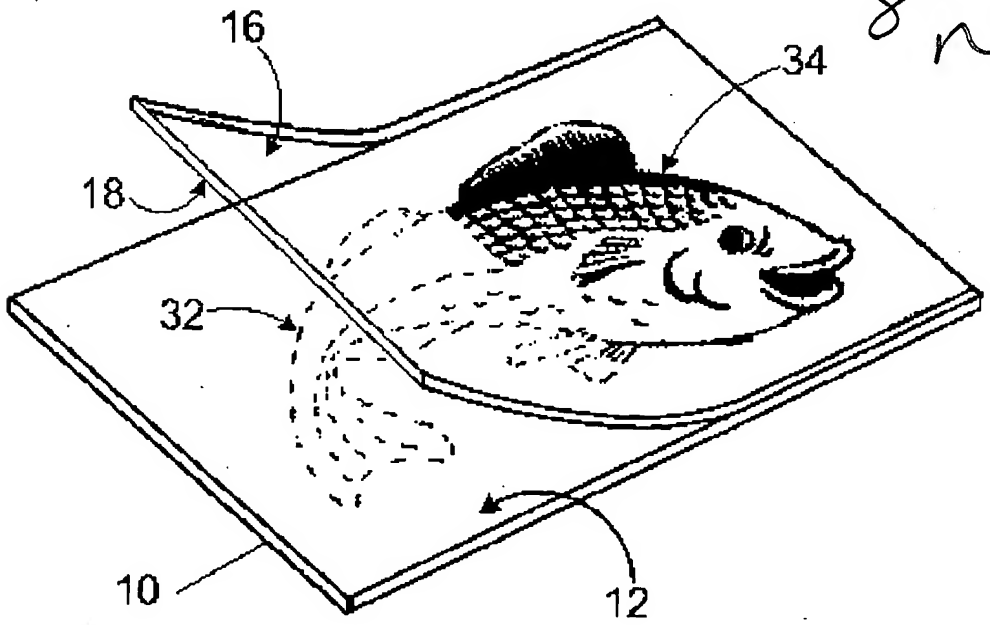
* cited by examiner

Primary Examiner—Daniel J. Colilla
Assistant Examiner—Marissa Ferguson-Samreth
(74) *Attorney, Agent, or Firm*—Luedeka, Neely & Graham, PC

(57) **ABSTRACT**

The invention relates to a device for developing latent or hidden images. The device includes a first substrate containing a hidden or latent image printed on a first surface thereof, wherein the image contains one or more chromogenic compounds. A second substrate having a first surface contains a developer coating for developing the hidden or latent image. The developer coating includes a developer compound, a micro-encapsulated solvent, and an adhesive. Upon sufficient interfacial contact between the first surfaces of the first and second substrates, a visible image is produced. The device enables production of a relatively vibrant, full color images which are substantially non-toxic and relatively inexpensive to make.

26 Claims, 2 Drawing Sheets



DOCUMENT-IDENTIFIER: US 7087265 B1
TITLE: Image developing device

CLAIMS:

10. The device of claim 1, wherein the chromogenic compound comprises a compound selected from the group consisting of crystal violet lactone, Michler's hydrol, fluoran compounds, leuco compounds, auramine compounds, bis-indolylphthalide compounds and combinations of two or more of the foregoing.

peracid

An acid containing a peroxide group (-O-OH); e.g., peracetic acid.

Synonym: peroxy acid.

(05 Mar 2000)

Previous: PepX dipeptidyl aminopeptidase, pequots, per-, peracephalus, peracetic acid
Next: peracute, peraeopod, perambulating ulcer, perameles, per anum

peracetic acid

<chemical> Ethaneperoxoic acid. A liquid that functions as a strong oxidizing agent. It has an acrid odour and is used as a disinfectant.

Pharmacological action: disinfectants.

Chemical name: Ethaneperoxoic acid

(12 Dec 1998)

Previous: PEP-UDP-GlcNAc transferase, PepX dipeptidyl aminopeptidase, pequots, per-, peracephalus
Next: peracid, peracute, peraeopod, perambulating ulcer, perameles, per anum

WEST Search History

Hide Items | Restore | Clear | Cancel

DATE: Wednesday, February 28, 2007

Hide?	Set Name	Query	Hit Count
		DB=PGPB,USPT,USOC,EPAB,JPAB,DWPI,TDBD; PLUR=YES; OP=OR	
☐	L1	((ammonia.ti.) and $sensor.ti.)	299
☐	L2	(((MICHLERS | MICHLERKETONE | MICHLERIS | MICHLERE | MICHLER | MICHLERI | MICHLERSKCTONE | MICHLERSKETONE)!) or ((MICHLERS | MICHLERKETONE | MICHLERIS | MICHLERE | MICHLER | MICHLERI | MICHLERSKCTONE | MICHLERSKETONE)!) or ((MICHLERS-KCTONE | MICHLER8)!) or ((MICHLER-KETONE)!) or ((MICHLER-KETONE)!) or ((MICHLER-KETONE | MICHLER-THIOKETONE)!) or ((MICHLER-KETONE | MICHLER/S)!) or ((MICHLER-KETONE | PARA-ROSANILINE | PARA-ROSANILINE-MONOSULPHONIC | PARA-ROSANILIN | PARA-ROSANIL | PARA-ROSANALINE | PARA-ROSALINE | PARA-ROSAIIILINE | PARA-ROSANILNE | PARA-ROSANILIN-DERIVATIVE | PARA-ROSANININE | PARA-ROSANI-LINE | PARA-ROSIIIILIN)!) or ((MICHLER-KETONE | PARA-ROSANILINE | PARA-ROSANILINE-MONOSULPHONIC | PARA-ROSANILIN | PARA-ROSANIL | PARA-ROSANALINE | PARA-ROSALINE | PARA-ROSAIIILINE | PARA-ROSANILNE | PARA-ROSANILIN-DERIVATIVE | PARA-ROSANININE | PARA-ROSANI-LINE | PARA-ROSIIIILIN)!) or ((MICHLER-KETONE | PARA-ROSANILINE | PARA-ROSANILINE-MONOSULPHONIC | PARA-ROSANILIN | PARA-ROSANIL | PARA-ROSANALINE | PARA-ROSALINE | PARA-ROSAIIILINE | PARA-ROSANILNE | PARA-ROSANILIN-DERIVATIVE | PARA-ROSANININE | PARA-ROSANI-LINE | PARA-ROSIIIILIN)!) or ((MICHLER-KETONE | PARAROSANILINE | PARAROSANILINEHYDROCHLORIDE | PARAROSANILINES | PARAROSANILINESULPHONIC | PARAROSANILINE-HYDROGEN | PARAROSANILINE-ETHANOL | PARAROSANILIN | PARAROSANILIIIE | PARAROSANILIDE | PARAROSANILI | PARAROSANILANE | PARAROSANILE | PARAROSANIL | PARAROSANIIINE | PARAROSANIHNE)!) or ((MICHLER-KETONE | ALPHA-NAPHTHOLAZOBENZENE | ALPHA-NAPHTHOLBENZEIN | ALPHA-NAPHTHOLBE | ALPHA-NAPHTHOLBENZALDEHYDEA | ALPHA-NAPHTHOLBENZENE | ALPHA-NAPHTHOLBENZIN | ALPHA-NAPHTHOLBENZOATE | ALPHA-NAPHTHOLBENZOIC | ALPHA-NAPHTHOLBENZYL)!) or ((MICHLER-KETONE | ALPHANAPHTHOL-BENZEIN)!) or ((MICHLER-KETONE | ALPHA-NAPHTHOL-BENZEIN | ALPHA-NAPHTHOL-BENZENE | ALPHA-NAPHTHOL-BENZOIN | ALPHA-NAPHTHOL-BENZEIIA | ALPHA-NAPHTHOL-BENZALDEHYDE-A)!) or ((MICHLER-KETONE | NAPHTHO-CHROME | NAPHTHOCHROME | NAPHTHOCHROM)!))	6351
☐	L3	l1 and l2	0

		DB=TDBD,DWPI,JPAB,EPAB,USOC,USPT,PGPB; PLUR=YES; OP=OR	
☐	L4	(MICHLERSHYDROL | MICHLERSHYDROL260 | MICHLERSHYDROL-260 | MICHLERS | MICHLERS-KCTONE | MICHLERSKETONE | MICHLERSKCTONE | MICHLER8)!	189
		DB=PGPB,USPT,USOC,EPAB,JPAB,DWPI,TDBD; PLUR=YES; OP=OR	
☐	L5	ammonia! near5 $sensor	878
☐	L6	L5 and (l2 or l4)	0
☐	L7	ammonia!	324490
☐	L8	L7 and (l2 or l4)	861
☐	L9	L8 and breath$	10
☐	L10	(l2 or l4).clm. same (devic$ or $device or apparatus or $apparatus).clm.	11
☐	L11	L10 not l9	9
☐	L12	l7 same balloon	269
☐	L13	L12.clm.	6
☐	L14	l12 and l5	2
☐	L15	(helicobacter or pylori or pyloris or pyloridis or pylor or hpylroi)	11172
☐	L16	L15 and (ammonia!.clm. or hyper-ammonemic$.clm. or hyperammonemic.clm.)	68
☐	L17	mh same (indicator or chromogen or color)	927
☐	L18	mh near20 (indicator or indicating or chromogen or color)	409
☐	L19	(pab or pararosaniline) near20 (indicator or indicating or chromogen or color)	118
☐	L20	(anb or alpha-naphtholbenzein or alphanaphtholbenzein or anaphtholbenzein) near20 (indicator or indicating or chromogen or color)	55
☐	L21	(neg or naphthochromegreen or naphtho-chrome-green or (naphthochrome near green)) near20 (indicator or indicating or chromogen or color)	27
☐	L22	L21 or l20 or l19 or l18 or l17 or l2	7541
☐	L23	L22 and (pylori or helicob or helicobacter or hpylori or pyloris or pyloridis or pylorum or urease)	36
☐	L24	L23 not l9	34

END OF SEARCH HISTORY

5	**Related Articles** for **PubMed** (Select **11440209**)	10:48:45 1
#2	Search **breath test device ammonia**	10:42:21
#1	Search **breath test device**	

R/S statement	R: ? S: ?
RTECS number	?
Supplementary data page	
Structure and properties	*n*, ε_r, etc.
Thermodynamic data	Phase behaviour Solid, liquid, gas
Spectral data	UV, IR, NMR, MS
Related compounds	
Other anions	?
Related ?	?
Related compounds	?
Except where noted otherwise, data are given for materials in their standard state (at 25 °C, 100 kPa) Infobox disclaimer and references	

Pararosaniline, Magenta 0, Basic Red 9, or **C.I. 42500** is a magenta dye having chemical formula $C_{19}H_{18}N_3Cl$. It is closely related to fuchsine, new fuchsine, and fuchsine acid. It makes the best Schiff's reagent.

See also

- fuchsine
- Schiff test

External links

- Links to external chemical sources

Retrieved from "http://en.wikipedia.org/wiki/Pararosaniline"

American Heritage Dictionary - *Cite This Source*

al·pha-naph·thol (āl'fə-nāf'thôl, -thōl, -nāp'-)

Pronunciation Key

n. An isomeric form of naphthol, $C_{10}H_7OH$, occurring as colorless or yellow prisms or powder, used in making dyes and perfumes and in organic synthesis.

```
?  s breath? (2n) detect? (2n) device?
Processing
       436343   BREATH?
      7126617   DETECT?
      2334100   DEVICE?
S10         62   S BREATH? (2N) DETECT? (2N) DEVICE?

?  s s10 and (chromagen? or dye? or color?)
           62   S10
          667   CHROMAGEN?
       690157   DYE?
      1579994   COLOR?
S11         10   S S10 AND (CHROMAGEN? OR DYE? OR COLOR?)

?  rd
S12          5   RD  (UNIQUE ITEMS)

?  t s12/ti/all
```

12/TI/1 (Item 1 from file: 155) **Links**

MEDLINE(R)

Breath ammonia testing for diagnosis of hepatic encephalopathy.

12/TI/2 (Item 1 from file: 34) **Links**
SciSearch(R) Cited Ref Sci

Preliminary observations on the Colibri CO2-indicator

12/TI/3 (Item 1 from file: 98) **Links**
General Sci Abs

A commercial device involving the breathalyzer test reaction.

Augmented Title: FINAL CALL

12/TI/4 (Item 1 from file: 135) **Links**
NewsRx Weekly Reports

Researchers from University of Washington publish latest findings

12/TI/5 (Item 2 from file: 135) **Links**
NewsRx Weekly Reports

Minimally invasive breath ammonia test not suitable for diagnosis

? pause
>>>I: PAUSE started, just enter a command to resume

? t s12/3,kwic/all
>>>W: KWIC option is not available in file(s): 399

12/3,KWIC/1 (Item 1 from file: 155) **Links**
Fulltext available through: USPTO Full Text Retrieval Options ScienceDirect
MEDLINE(R)

19417257 **PMID:** 16187173
Breath ammonia testing for diagnosis of hepatic encephalopathy.

DuBois Suja; Eng Sue; Bhattacharya Renuka; Rulyak Steve; Hubbard Todd; Putnam David; Kearney David J
Department of Medicine, Division of Gastroenterology, University of Washington School of Medicine, Seattle, Washington 98108, USA.
Digestive diseases and sciences (United States) Oct 2005 , 50 (10) p1780-4 , **ISSN:** 0163-2116--Print **Journal Code:** 7902782
Publishing Model Print
Document type: Clinical Trial; Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: MEDLINE; Completed
...this study was to evaluate the ability of a minimally invasive, highly sensitive optical sensing **device** to **detect** ammonia in the **breath** of patients with end-stage liver disease and to evaluate the correlation of breath ammonia... ...an apparatus that consists of a sensor (a thin membrane embedded with a pH-sensitive **dye**) attached to a fiberoptic apparatus that detects optical absorption. Helicobacter pylori testing was performed using...

12/3,KWIC/2 (Item 1 from file: 34) **Links**
Fulltext available through: USPTO Full Text Retrieval Options ScienceDirect
SciSearch(R) Cited Ref Sci

07221576 **Genuine Article#:** 138QM **No. References:** 64
Preliminary observations on the Colibri CO2-indicator

Author: Petroianu GA (REPRINT) ; Maleck WH; Bergler WF; Altmannsberger S; Rufer R
Corporate Source: UNIV HEIDELBERG,FAK KLIN MED MANNHEIM, INST PHARMAKOL & TOXIKOL, MAYBACHSTR 14-16/D-68169 MANNHEIM//GERMANY/ (REPRINT); UNIV HEIDELBERG,INST PHARMACOL/D-6800 MANNHEIM//GERMANY/
Journal: AMERICAN JOURNAL OF EMERGENCY MEDICINE , 1998 , V 16 , N7 (NOV) , P 677-680
ISSN: 0735-6757 **Publication date:** 19981100
Publisher: W B SAUNDERS CO , INDEPENDENCE SQUARE WEST CURTIS CENTER, STE 300, PHILADELPHIA, PA 19106-3399
Language: English **Document Type:** ARTICLE (ABSTRACT AVAILABLE)
Abstract: The performance of a new **colorimetric** CO2-indicator (Colibri) was assessed in mini pigs. It performed well during 8 hour procedures... ...of drugs (epinephrine, atropine, lidocaine, and naloxone) interfered with its function. It gave a distinct **color** change at high ventilation frequencies up to 120/min. The only problem observed was difficulty in matching the **colors** displayed with the comparison **color** chart provided, The Colibri's performance seems at least equal to that of the EasyCAP...
Identifiers-- ...TIDAL CARBON-DIOXIDE; ENDOTRACHEAL-TUBE PLACEMENT; **COLORIMETRIC BREATH** INDICATOR; ESOPHAGEAL **DETECTOR DEVICE**; HOSPITAL CARDIAC-ARREST; CO2 DETECTOR; CARDIOPULMONARY-RESUSCITATION; EMERGENCY INTUBATION; PARAMEDICS; VERIFICATION

12/3,KWIC/3 (Item 1 from file: 98) **Links**
General Sci Abs

03258833 **H.w. Wilson Record Number:** BGSA96008833
A commercial device involving the breathalyzer test reaction.

Augmented Title: FINAL CALL
Dombrink, Kathleen J
Journal of Chemical Education (J Chem Educ) v. 73 (Feb. 1996) p. 135-6
Special Features: bibl il **ISSN:** 0021-9584
Language: English
Country Of Publication: United States

Abstract: ...the orange dichromate ion Cr2O72- to the green chromium(III) ion Cr3+ by alcohol to **detect** alcohol in the **breath**. The **device** consists of a 10 cm tube containing 3 bands of silica gel crystals coated with acidic potassium dichromate solution, separated from each other by an inert **dye** material. Two plugs of CoCl2-coated silica gel at either end, which are discarded when... ...breath to flow through the tube for 60 seconds. The number of bands that change **color** and the intensity of the **color** change indicate the relative amount of alcohol in the breath

12/3,KWIC/4 (Item 1 from file: 135) **Links**
NewsRx Weekly Reports

0000303924 (USE FORMAT 7 OR 9 FOR FULLTEXT)

Researchers from University of Washington publish latest findings

Obesity, Fitness & Wellness Week, May 30, 2006, p.2679

DOCUMENT TYPE: Expanded Reporting LANGUAGE: English
RECORD TYPE: FULLTEXT

Word Count:
1243

... University of Washington evaluated both "the ability of a minimally invasive, highly sensitive optical sensing **device** to **detect** ammonia in the **breath** of patients with end-stage liver disease," and "the correlation of breath ammonia levels, arterial...

...an apparatus that consists of a sensor (a thin membrane embedded with a pH-sensitive **dye**) attached to a fiberoptic apparatus that detects optical absorption."

" Helicobacter pylori testing was performed using...

12/3,KWIC/5 (Item 2 from file: 135) **Links**
NewsRx Weekly Reports

0000258590 (USE FORMAT 7 OR 9 FOR FULLTEXT)

Minimally invasive breath ammonia test not suitable for diagnosis

Life Science Weekly, November 22, 2005, p.592

DOCUMENT TYPE: Expanded Reporting LANGUAGE: English
RECORD TYPE: FULLTEXT

Word Count:
403

... University of Washington evaluated both "the ability of a minimally invasive, highly sensitive optical sensing **device** to **detect** ammonia in the **breath** of patients with end-stage liver disease," and "the correlation of breath ammonia levels, arterial...

...an apparatus that consists of a sensor (a thin membrane embedded with a pH-sensitive **dye**) attached to a fiberoptic apparatus that detects optical absorption."

" Helicobacter pylori testing was performed using...

[0006] Another method for the detection of H. pylori infection requires collecting gas in the gastric cavity, and detecting in this gas ammonia and organic amines that are generated due to activities of the bacilli (see, e.g., U.S. Pat. No. 6,312,918). In this method, gas from the gastric cavity is led into the oral cavity by generating a vomiting-reflex, and the gas is collected by means of a metering suction pump which causes the gas to flow through a detection tube which changes color when ammonia and organic amines are present. Again, however, this technique is invasive, causes discomfort to the patient and is relatively expensive to perform.

: Br J Biomed Sci. 2001;58(2):66-75.

Links

Ammonia vapour in the mouth as a diagnostic marker for Helicobacter pylori infection: preliminary 'proof of principle' pharmacological investigations.

Dun CDR, Blac M, Cowell DC, Penaul C, Ratcliffe NM, Spence R, Teare C.

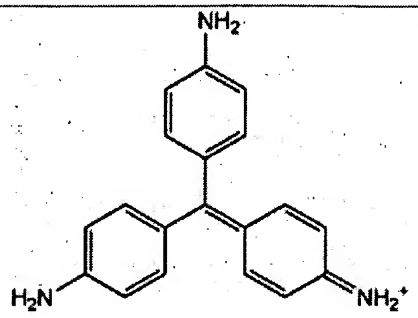
Faculty of Applied Sciences and Faculty of Health and Social Care, University of the West of England, Bristol, UK.
chrisann@dunn9950.freeserve.co.uk

Most current non-invasive tests for Helicobacter pylori depend on the conversion of labelled (13C or 14C) urea to labelled carbon dioxide (13CO2 or 14CO2) and ammonium (NH4+) by the enzyme urease, with the labelled CO2 detected in exhaled air. Despite suggestions going back over a number of years, the alternative possibility of using NH4+ (in the form of gaseous ammonia [NH3]) as the test parameter has received little or no attention. However, this approach is now being explored using a chemiresistive sensor detecting sub-parts per million concentrations of NH3. An in vitro 'glass stomach' (containing various volumes of hydrochloric acid [HCl] and ammonium chloride [NH4Cl]) was used to evaluate the means of increasing 'gastric' pH to that of the NH4+-->NH3 transition that occurs significantly at pH 9.24. This 'stomach' also was used to study mechanisms by which NH3 may be expelled in a pulse (as a surrogate belch), either by the in situ production of CO2 or through an exogenous source. On the basis of the protocols developed, H. pylori-negative subjects were tested before and after ingestion of 10 mg NH4Cl (as a surrogate for bacteria-produced NH4,), and H. pylori-positive subjects were tested without taking urea or NH4Cl. 'Intragastric' pH in the in vitro 'glass stomach' could be increased above pH 9.24 by adding a mixture of 15-30 mL magnesium hydroxide mixture (or the proprietary equivalent) and 50 mL water, and the resulting NH3 expelled by adding 100 mL CO2-saturated cold water (sparkling water). In vivo, NH3 levels in the oral cavity of H. pylori-negative subjects were increased after ingestion of 10 mg NH4Cl; however, levels in the oral cavity of a small number of H. pylori-positive subjects were two- to threefold higher after magnesium hydroxide and sparkling water. On the basis of in vitro studies, an in vivo protocol was developed to increase gastric pH above that required for the NH4+-->NH3 transition, and a mechanism established to release the NH3 into the oral cavity. Preliminary in vivo data confirm the chemiresistive sensor is sufficiently sensitive to NH3 to distinguish H. pylori-negative subjects who have taken 10 mg NH4Cl from those who have not, and clearly distinguish H. pylori-negative subjects from H. pylori-positive subjects. Ingestion of urea or other labelled tracers is not required, nor is belching; and the sensor takes less than two minutes to reach a maximum response. The data provide good evidence that the chemiresistive detection of NH3 has considerable potential as a rapid, point-of-care diagnostic test for H. pylori infection.

PMID: 11440209 [PubMed - indexed for MEDLINE]

Pararosaniline

From Wikipedia, the free encyclopedia

Pararosanilin	
 Chloride counteranion not displayed	
General	
Systematic name	Benzenamine, 4- [(4-aminophenyl) (4-imino-2,5-cyclohexadien-1-ylidene) methyl]-, monohydrochloride
Other names	■ Pararosaniline hydrochloride ■ Pararosaniline Chloride ■ C.I. 42500 ■ C.I. Basic Red 9, Monohydrochloride ■ Para Magenta
Molecular formula	$C_{19}H_{18}ClN_3$
SMILES	[Cl-].[NH2+]=C\1/C=C\C(/C=C/1)= C(/c2ccc(N)cc2)c3ccc(N)cc3
Molar mass	323.82 g/mol
Appearance	Green crystalline solid
CAS number	[569-61-9]
Properties	
Density and phase	? g/cm^3, ?
Solubility in water	slightly soluble
Melting point	268-270°C (541-543 K) dec.
Boiling point	?°C (? K)
Acidity (pK_a)	?
Structure	
Crystal structure	?
Dipole moment	? D
Hazards	
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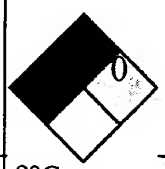
Main hazards	?
NFPA 704	
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DOCUMENT-IDENTIFIER: US 7052854 B2
** See image for Certificate of Correction **
TITLE: Application of nanotechnology and sensor technologies for ex-vivo diagnostics

PRIOR-PUBLICATION:

DOC-ID	DATE
US 20060040318 A1	February 23, 2006

Other Reference Publication (9):
Ganga-Zandzou, P.S. et al. "A 13C-urea breath test in children with Helicobacter pylori infection: validity of the use of a mask to collect exhaled breath sample," Acta. Paediatr. (2001), vol. 90, pp. 232-233. cit- ed by other

Other Reference Publication (17):
Perri, F. "Diagnosis of Helicobacter pylori infection: which is best? The urea breath test," Dig. Liver. Dis. (2000), vol. 32, Supp. 3, pp. S196-198. cited by other

CLAIMS:

6. The method according to claim 1, wherein the target analyte/biomarker is selected from the group consisting of acetaldehyde, acetone, ammonia, CO, chloroform, dichlorobenzene, diethylamine, hydrogen, isoprene, methanethiol, methylethylketone, O-toluidine, pentane sulfides and sulfides, H.sub.2S, MES, and Me.sub.2S.

L15: Entry 1 of 1 File: USPT May 30, 2006

DOCUMENT-IDENTIFIER: US 7052854 B2
**** See image for Certificate of Correction ****
TITLE: Application of nanotechnology and sensor technologies for ex-vivo diagnostics

PRIOR-PUBLICATION:

DOC-ID	DATE
US 20060040318 A1	February 23, 2006

Brief Summary Text (6):
Aptamers have recently been identified as potentially effective biosensors for molecules and compounds of scientific and commercial interest (see Brody, E. N. and L. Gold, "Aptamers as therapeutic and diagnostic agents," J. Biotechnol., 74(1):5 13 (2000) and Brody et al., "The use of aptamers in large arrays for molecular diagnostics," Mol. Diagn., 4(4):381 8 (1999)). For example, aptamers have demonstrated greater specificity and robustness than antibody-based diagnostic technologies. In contrast to antibodies, whose identification and production completely rest on animals and/or cultured cells, both the identification and production of aptamers takes place in vitro without any requirement for animals or cells.

Brief Summary Text (68):
"Track-etch" polymeric or porous alumina membranes can be used in the preparation of nanotubes. Track-etch membranes prepared from polycarbonate and polyester are available from suppliers such as Osmonics (Minnetonka, Minn.) and Whatman (Maidstone, Kent UK). Tracl-etch membranes contain randomly distributed cylindrical pores of uniform diameter than run through the entire thickness of the membrane. Pore diameters as small as 10 nm are commercially available at pore densities of up to 10.sup.9 pores per square centimeter.

Brief Summary Text (69):
Porous alumina membranes, which are commercially available from Whatman (Maidstone, Kent UK), are prepared electronically from aluminum metal. Pore diameters as small as 5 nm can be achieved at pore densities as high as 10.sup.11 pores per square centimeter. Membranes can be prepared having the membrane thickness from as small as 100 nm to as large as 100 .mu.m.

Brief Summary Text (108):
In another embodiment, the invention uses fluid sensor technology, such as commercial devices known as "artificial noses," "electronic noses," or "electronic tongues." These devices are capable of qualitative and/or quantitative analysis of simple or complex gases, vapors, odors, liquids, or solutions. A number of patents and patent applications which describe fluid sensor technology include the following: U.S. Pat. Nos. 5,945,069; 5,918,257; 5,891,398; 5,830,412; 5,783,154; 5,756,879; 5,605,612; 5,252,292; 5,145,645; 5,071,770; 5,034,192; 4,938,928; and 4,992,244; and U.S. patent application Ser. No. 2001/0050288. Certain sensitive, commercial off-the-shelf electronic noses, such as those provided by Cyrano

Sciences, Inc. ("CSI") (i.e., CSI's portable Electronic Nose and CSI's Nose-Chip.TM. integrated circuit for odor-sensing--U.S. Pat. No. 5,945,069), can be used in the present invention to detect the presence of surrogate markers in bodily fluid samples.

Previous Doc Next Doc Go to Doc#

US 20040077093A1

(19) **United States**

(12) **Patent Application Publication** (10) **Pub. No.: US 2004/0077093 A1**

Pan (43) **Pub. Date: Apr. 22, 2004**

(54) **METHOD AND APPARATUS FOR THE DETECTION OF THE PRESENCE OF A BACTERIA IN THE GASTROINTESTINAL TRACT OF A SUBJECT**

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Deerfield, IL 60015-4633 (US)

(73) Assignees: **Baxter International Inc.; Baxter Healthcare S.A.**

(21) Appl. No.: **10/617,008**

(22) Filed: **Jul. 10, 2003**

Related U.S. Application Data

(60) Provisional application No. 60/395,268, filed on Jul. 12, 2002.

Publication Classification

(51) **Int. Cl.**[7] **C12Q 1/04**

(52) **U.S. Cl.** **436/37**; 435/34

(57) **ABSTRACT**

The present invention generally relates to a method and an apparatus to detect the presence of a bacteria in a subject. More particularly, the method is directed toward the detection of a bacteria which is associated with catalyzing urea to carbon dioxide and ammonia when present in the gastrointestinal tract of a subject. The method comprises administering urea to a subject, obtaining a fluid sample from the subject after the administration of the urea and then determining the presence or amount of ammonia gas in the fluid sample. The apparatus facilitates ease of implementing the method and facilitates accuracy and speed of obtaining the test result.

Kit
Balloon
surface
beads

Kit

FIG. 1

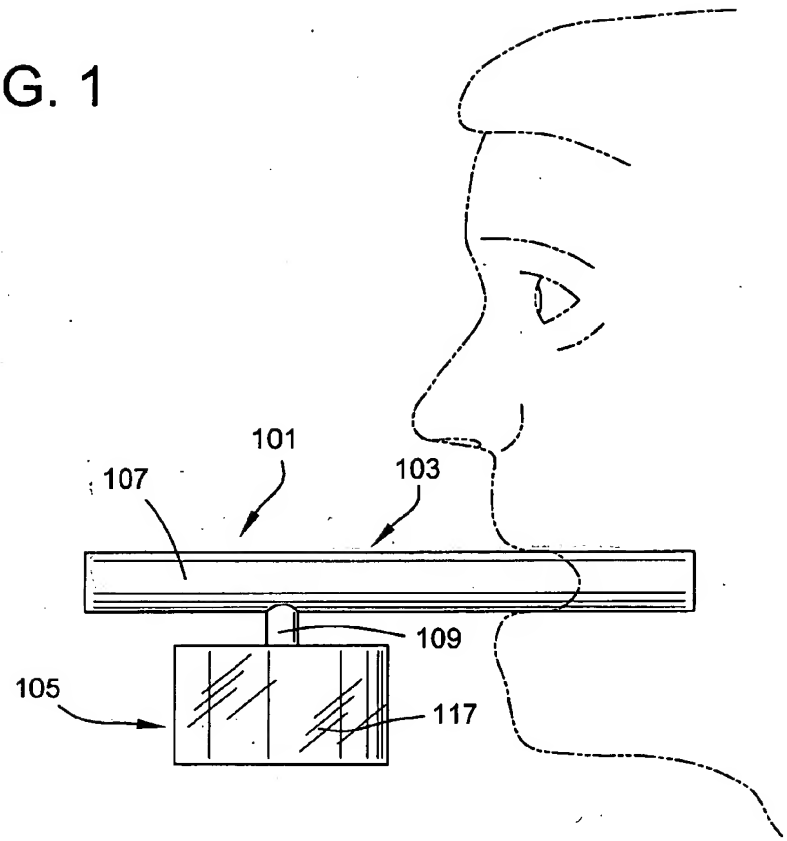
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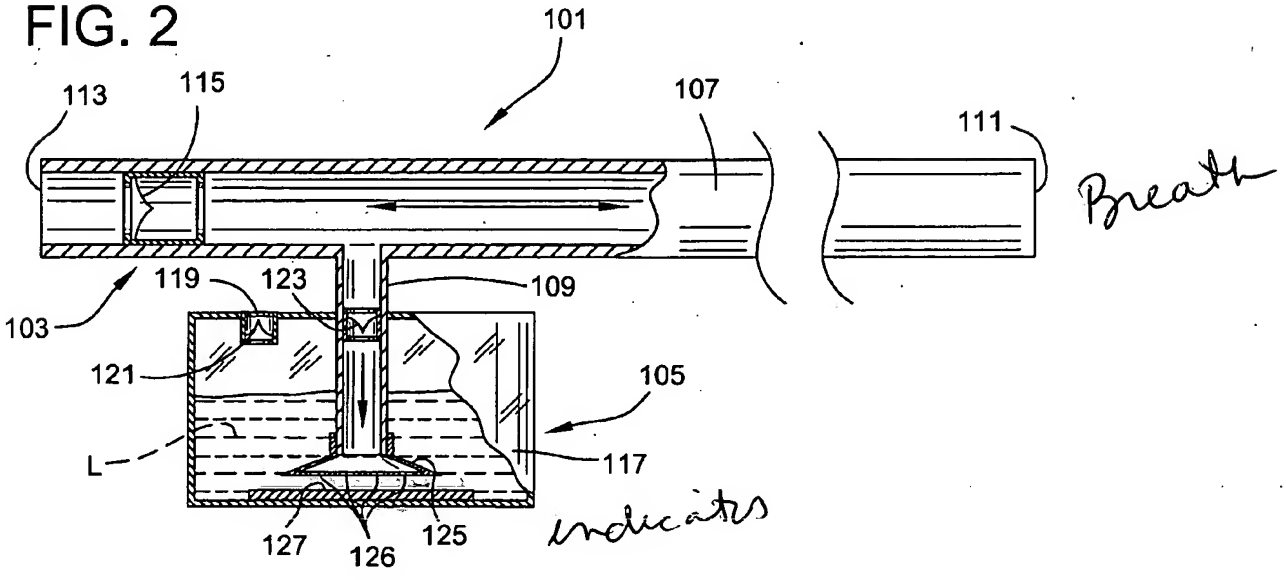
FIG. 2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Indicators

FIG. 3

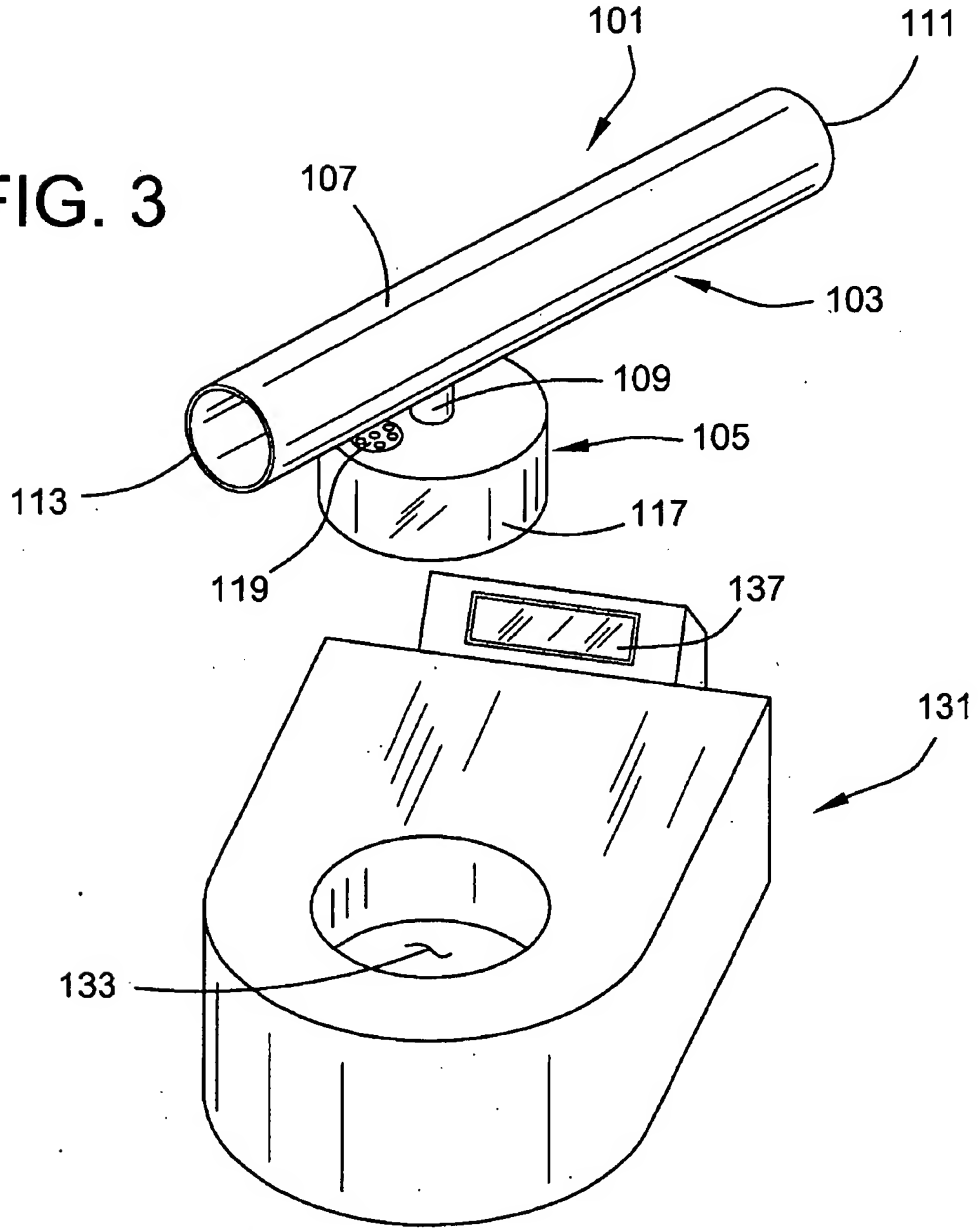
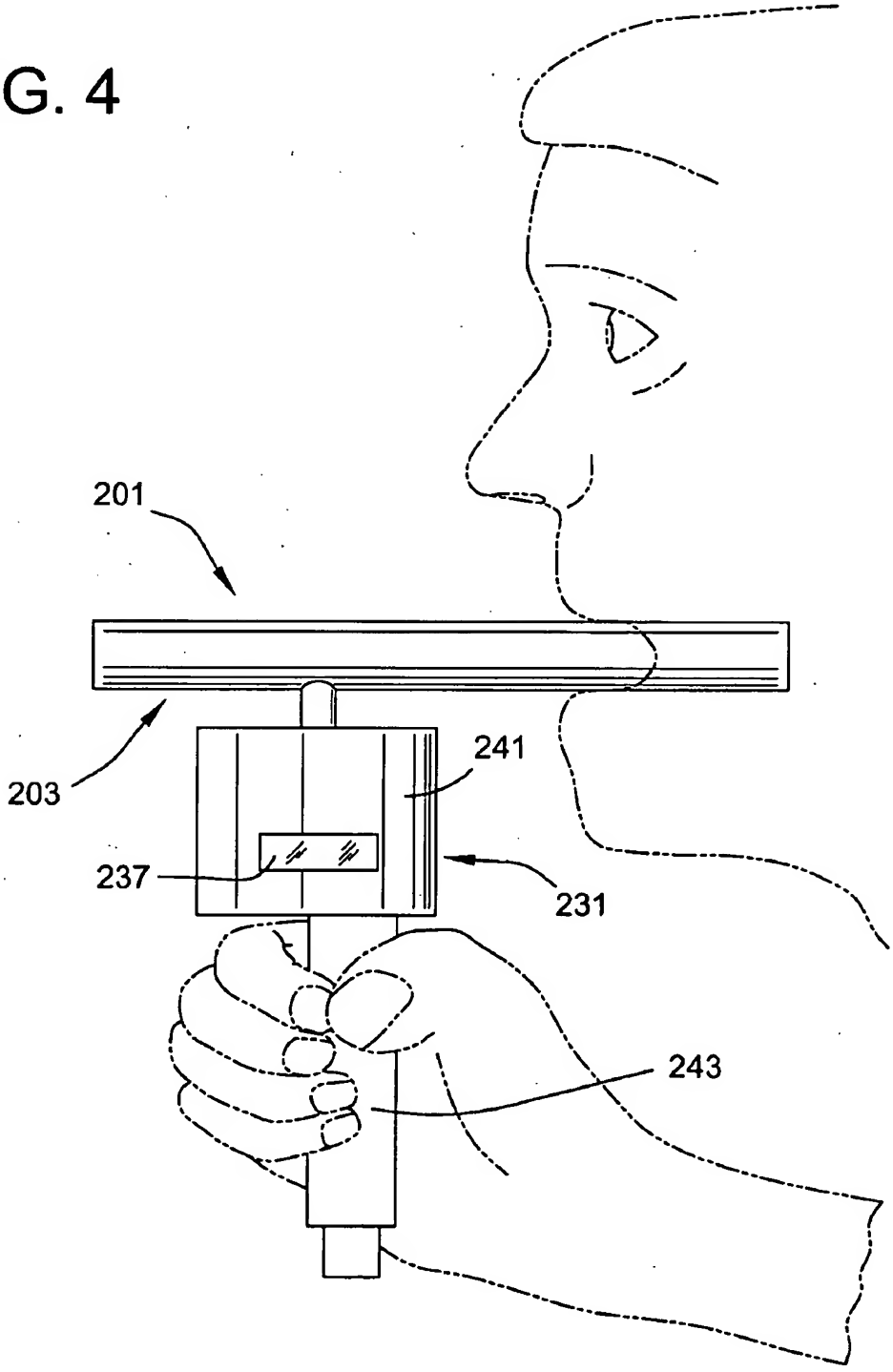


FIG. 4



US 20040077093A1

(19) United States

(12) Patent Application Publication (10) Pub. No.: US 2004/0077093 A1

Pan (43) Pub. Date: Apr. 22, 2004

(54) METHOD AND APPARATUS FOR THE DETECTION OF THE PRESENCE OF A BACTERIA IN THE GASTROINTESTINAL TRACT OF A SUBJECT

(75) Inventor: Lee Pan, Tampa, FL (US)

Correspondence Address:
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Corporate Counsel, Renal Division
Baxter Healthcare Corporation
One Baxter Parkway
Deerfield, IL 60015-4633 (US)

(73) Assignees: Baxter International Inc.; Baxter Healthcare S.A.

(21) Appl. No.: 10/617,008

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Publication Classification

(51) Int. Cl.[7] C12Q 1/04

(52) U.S. Cl. 436/37; 435/34

(57) ABSTRACT

The present invention generally relates to a method and an apparatus to detect the presence of a bacteria in a subject. More particularly, the method is directed toward the detection of a bacteria which is associated with catalyzing urea to carbon dioxide and ammonia when present in the gastrointestinal tract of a subject. The method comprises administering urea to a subject, obtaining a fluid sample from the subject after the administration of the urea and then determining the presence or amount of ammonia gas in the fluid sample. The apparatus facilitates ease of implementing the method and facilitates accuracy and speed of obtaining the test result.

FIG. 1

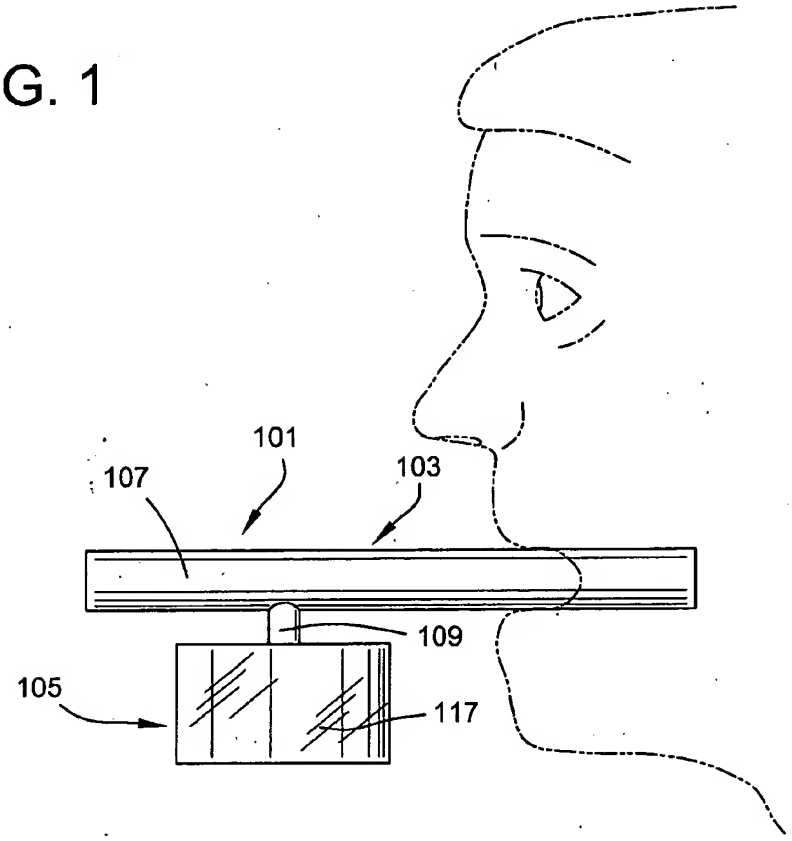
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FIG. 2

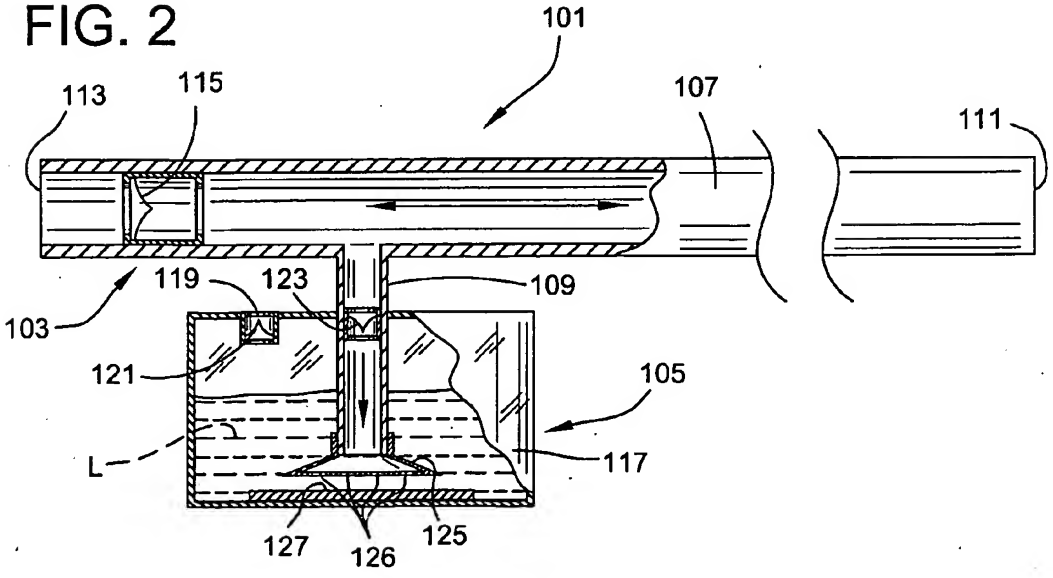
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FIG. 3

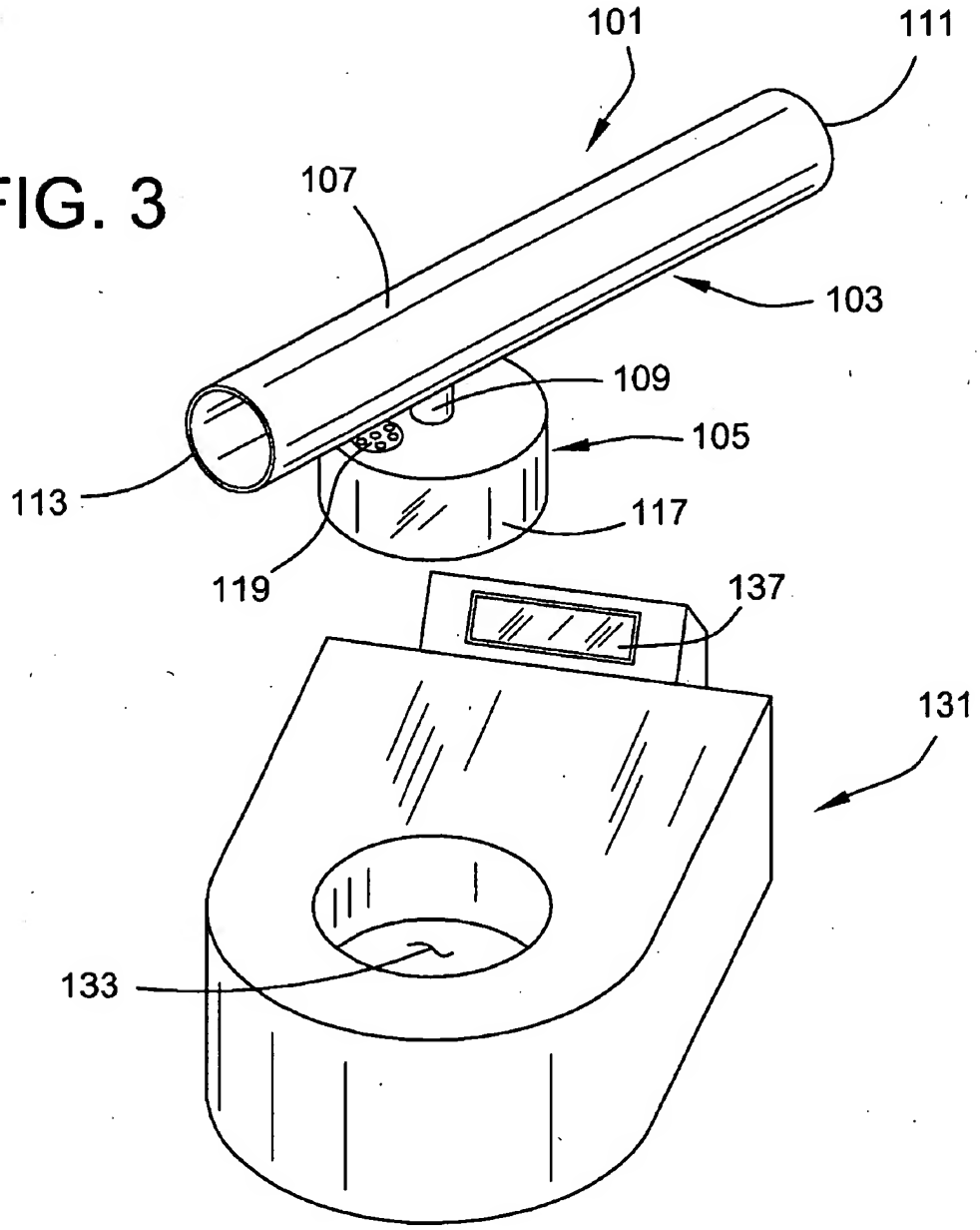
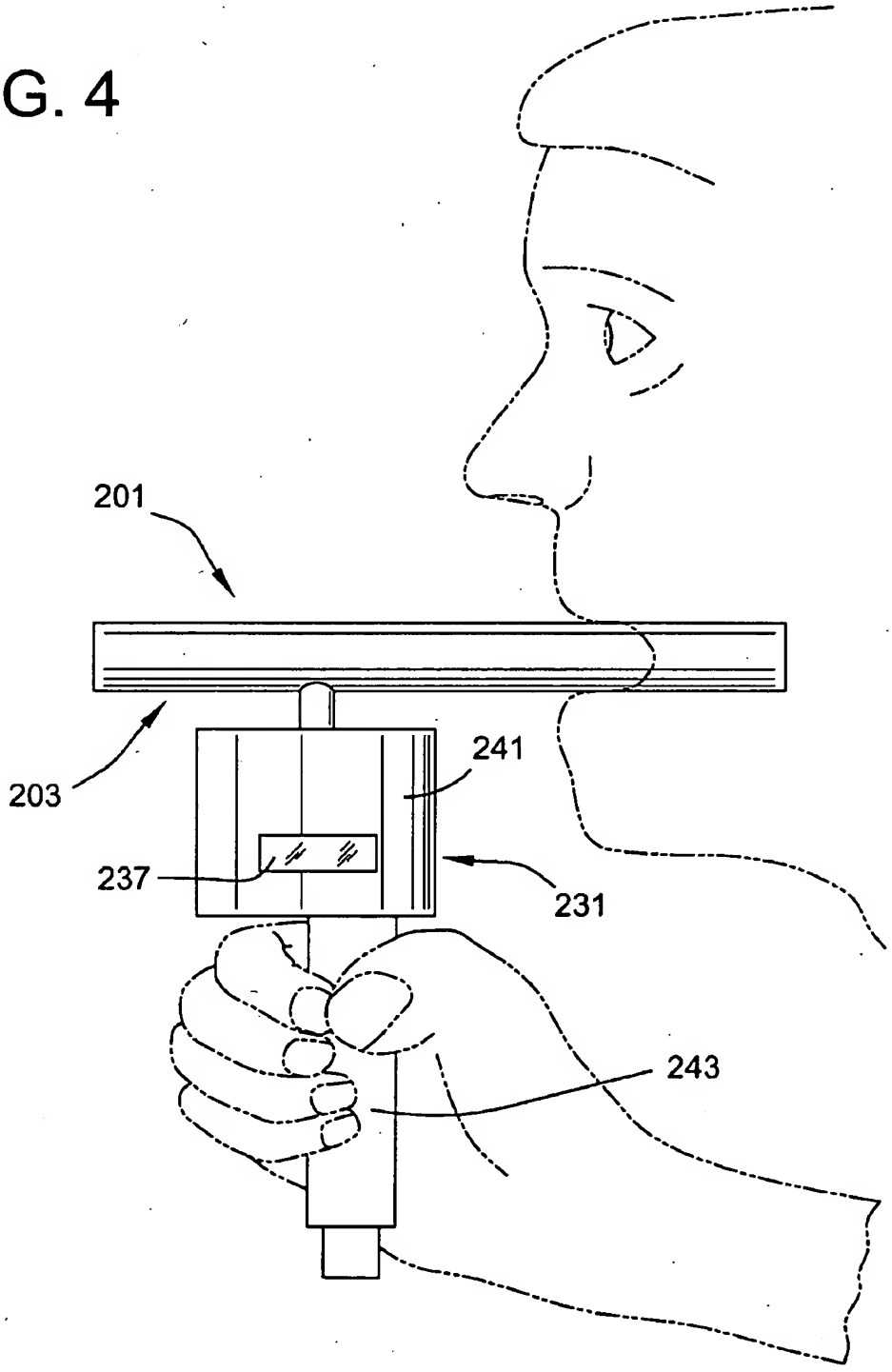


FIG. 4



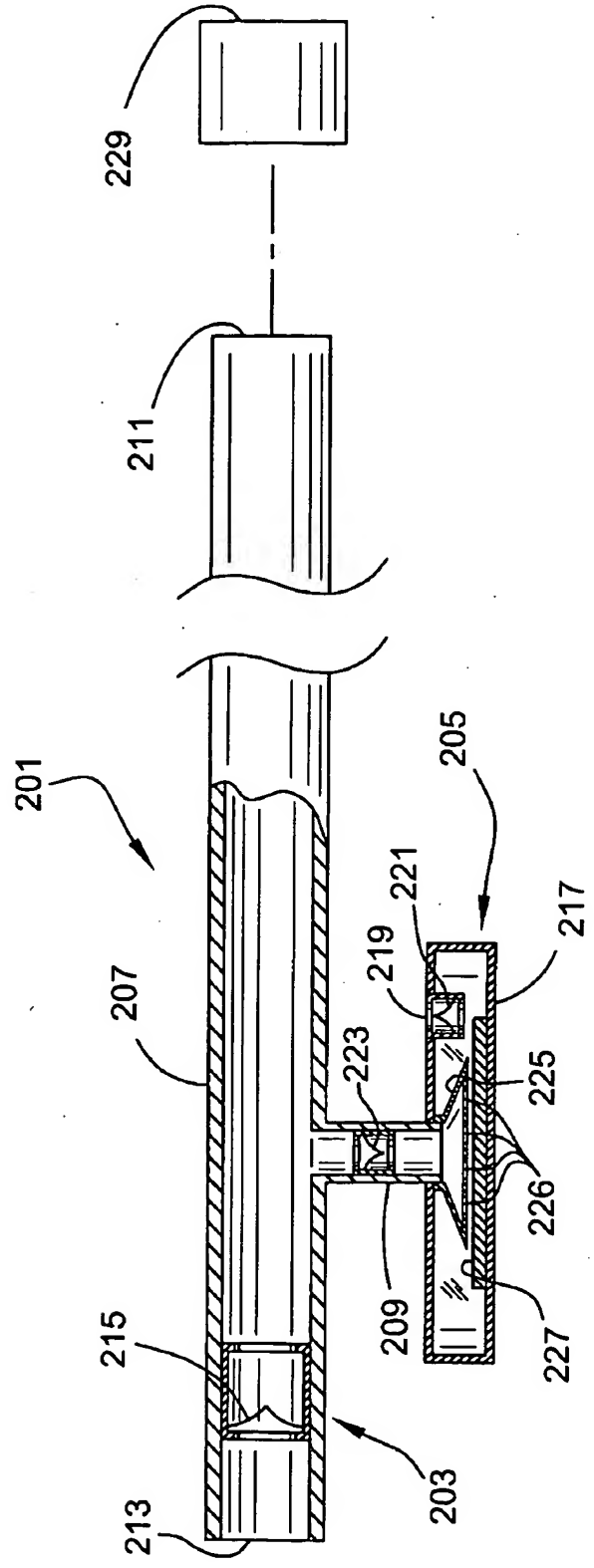
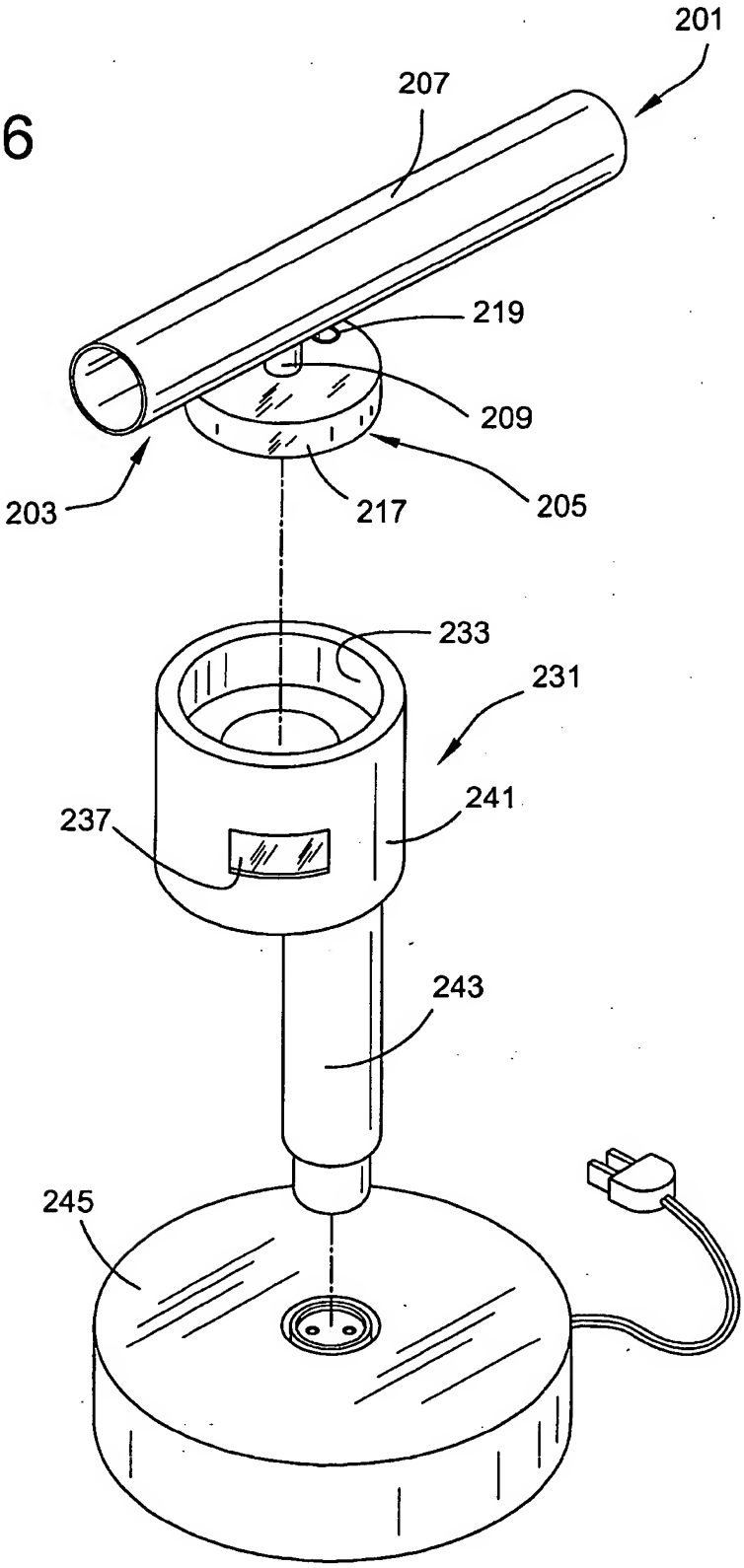
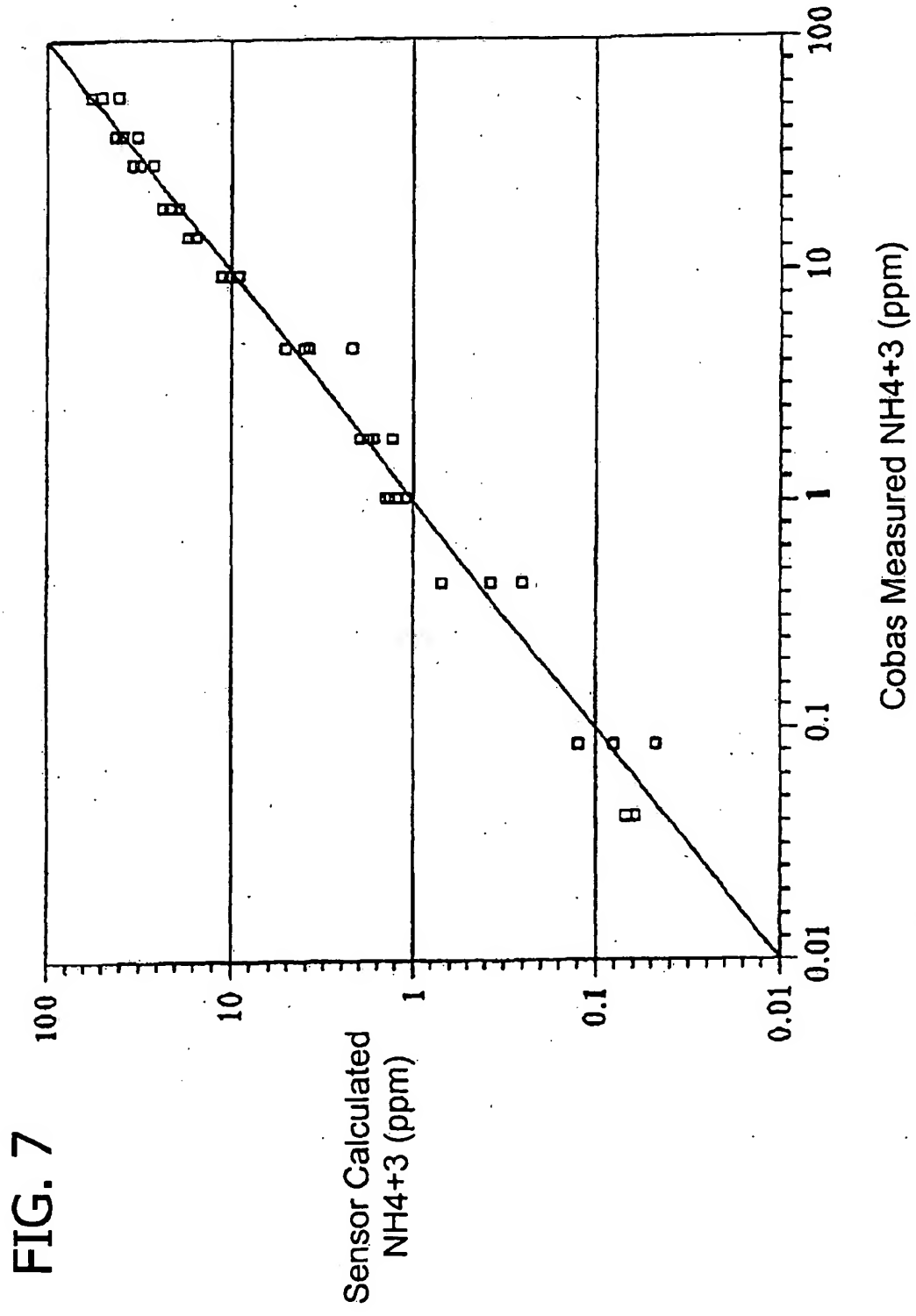


FIG. 5

FIG. 6




100
10
1
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Sensor Calculated NH4+3 (ppm)
100
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1
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0.01
Cobas Measured NH4+3 (ppm)

FIG. 7

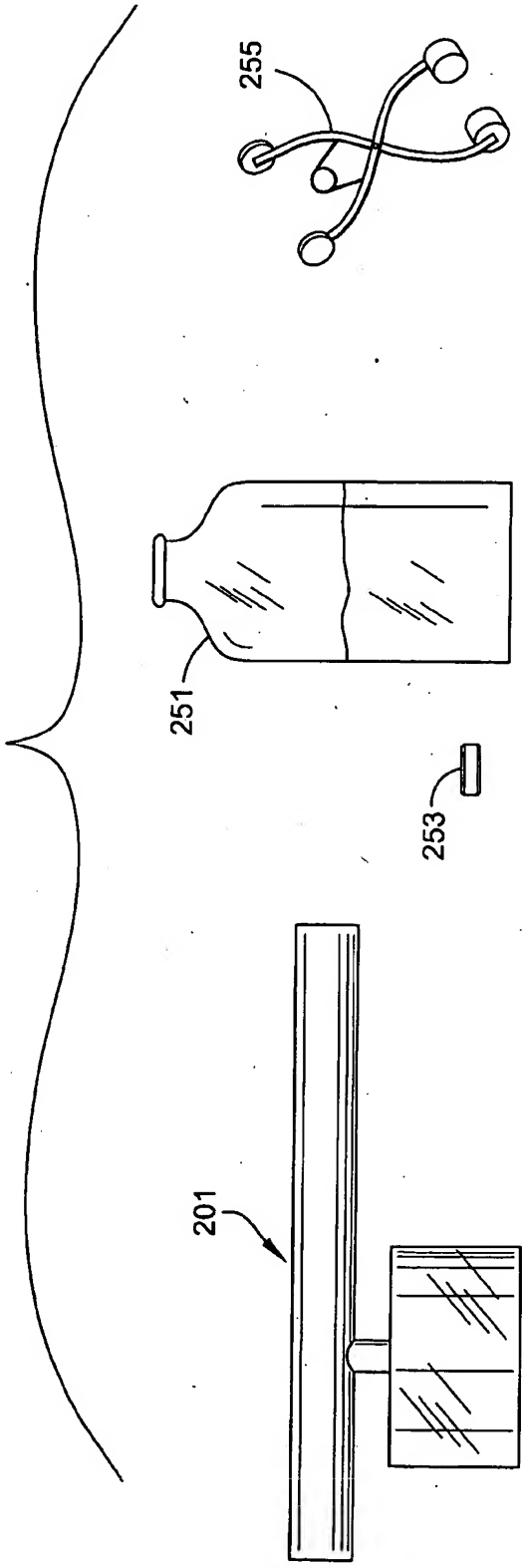


FIG. 8

FIG. 9

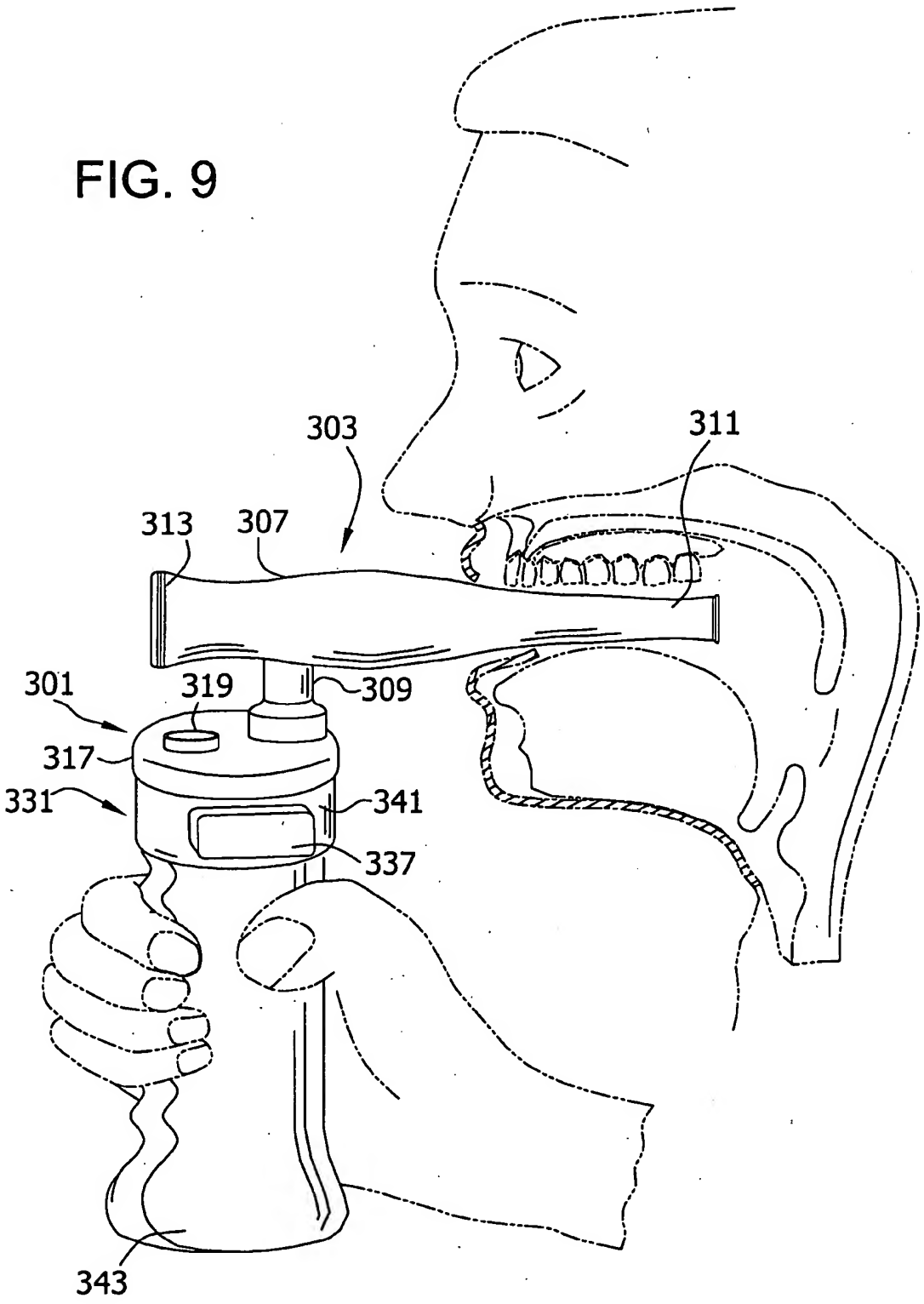
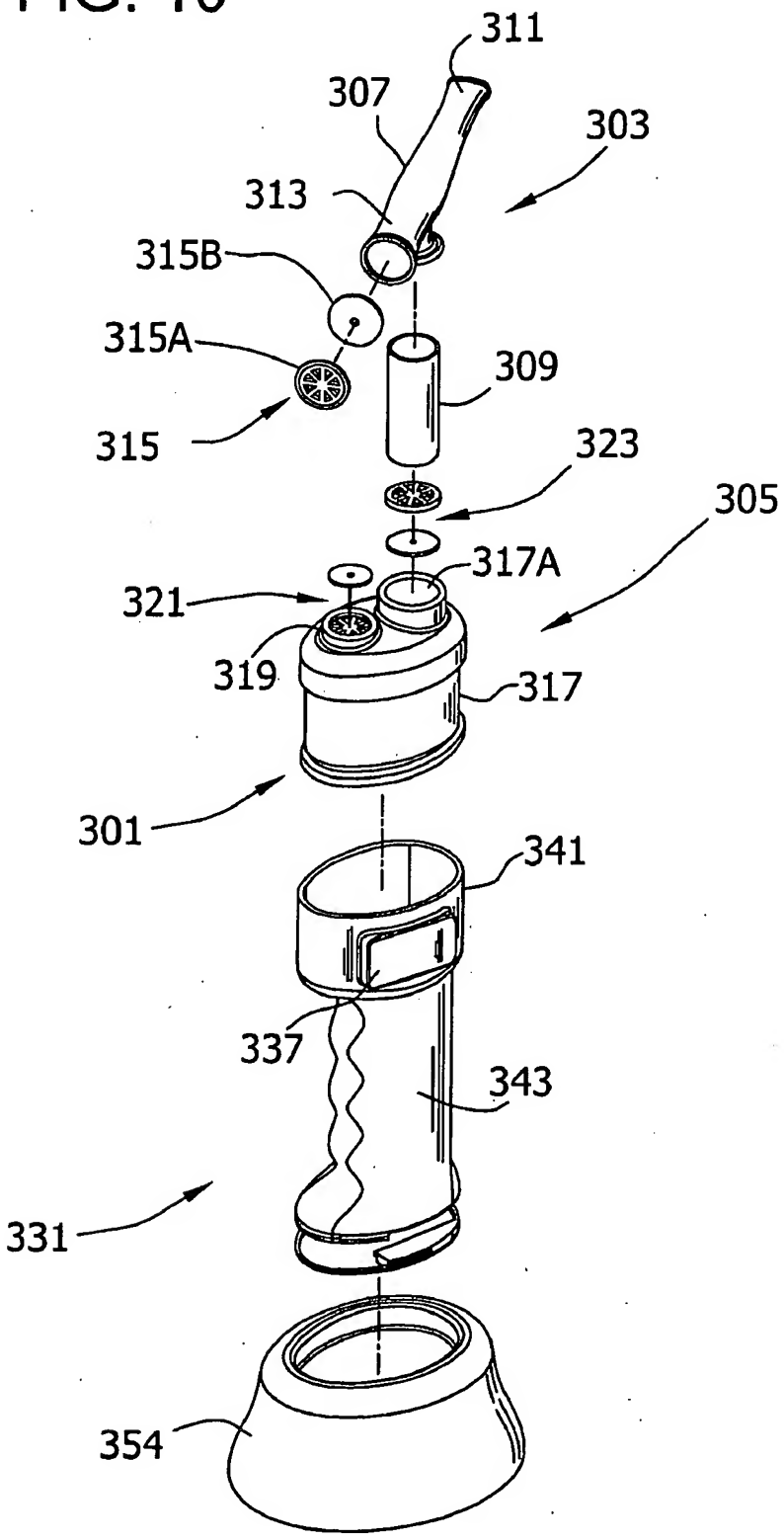


FIG. 10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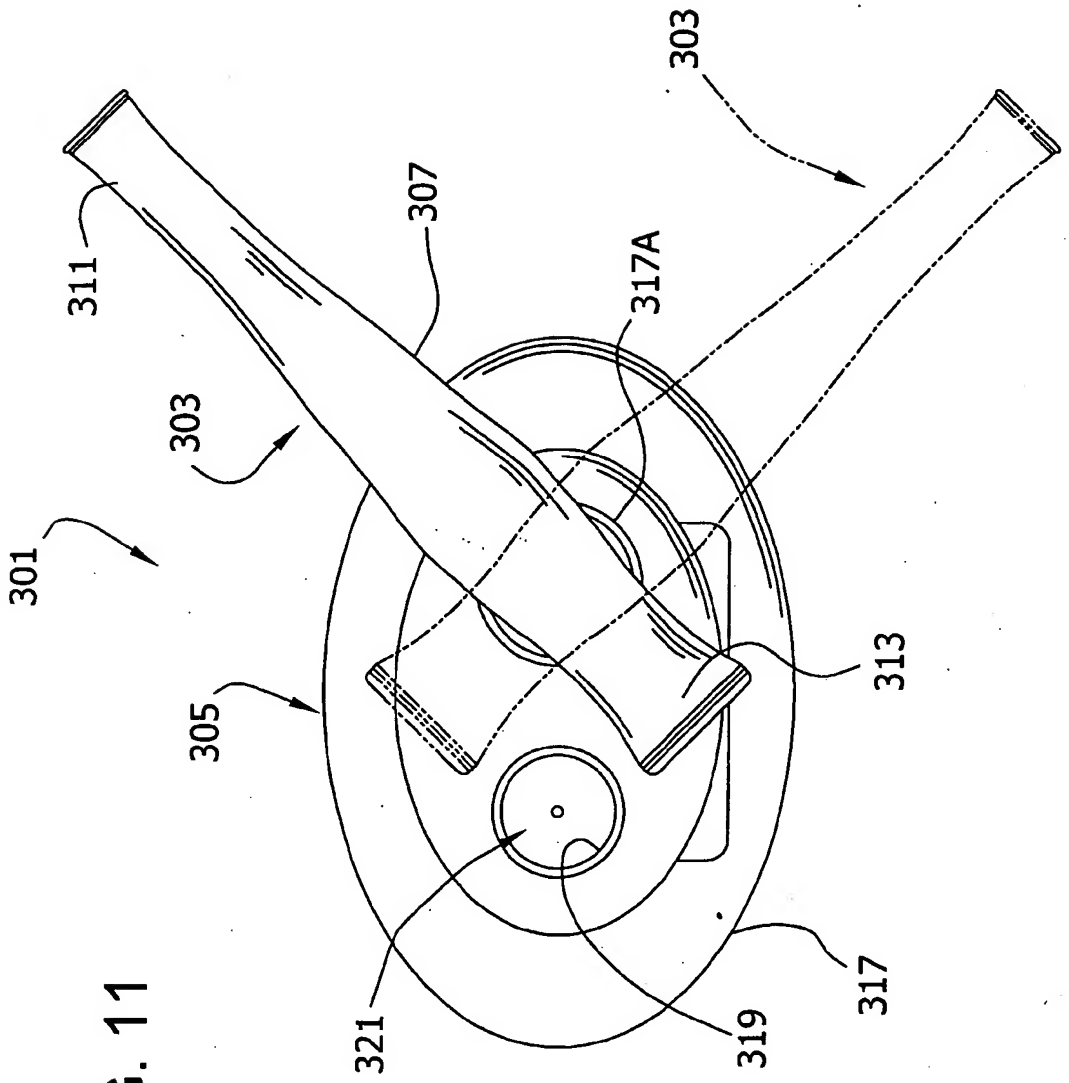

FIG. 11

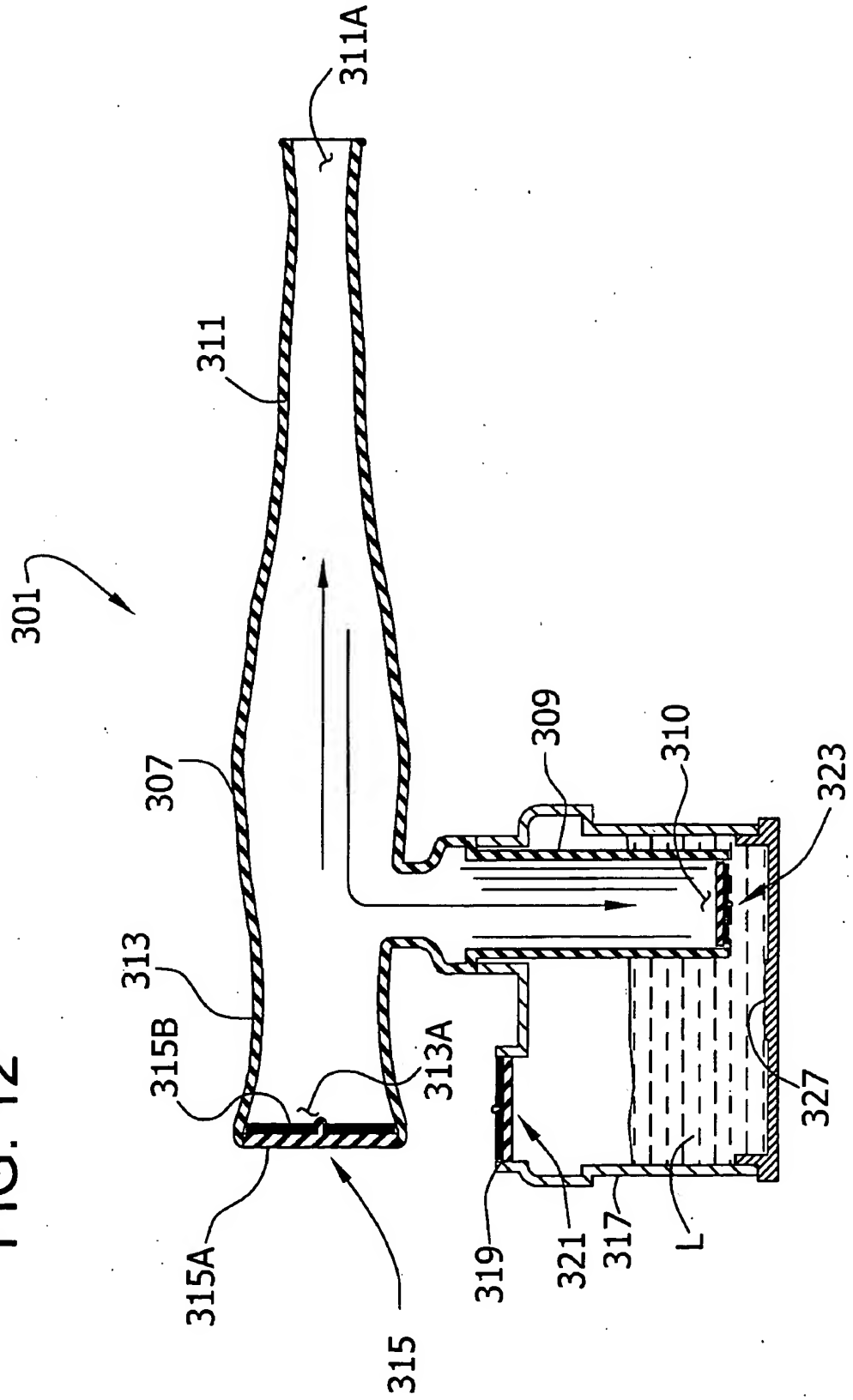
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FIG. 12

METHOD AND APPARATUS FOR THE DETECTION OF THE PRESENCE OF A BACTERIA IN THE GASTROINTESTINAL TRACT OF A SUBJECT

FIELD OF THE INVENTION

[0001] The present invention generally provides a method and an apparatus for the detection of a bacteria in a subject. More particularly, the method is directed to the detection of a bacteria associated with the catalytic breakdown of urea to carbon dioxide and ammonia in the gastrointestinal tract of a subject.

BACKGROUND OF THE INVENTION

[0002] Gastrointestinal associated disorders are an important public health concern. In the United States alone, approximately twenty-five million Americans suffer from peptic ulcer disease with an annual incremental 500,000 to 800,000 new cases. Additionally, costs associated with treating these disorders are considerable.

[0003] Until recently, excessive gastric acidity and mental stress were thought to be the major pathophysiological reasons for the occurrence of gastrointestinal disorders. In 1982, however, a new spiral Gram-negative bacterium, later to be named Helicobacter pylori, was isolated from the gastric mucosa of significant numbers of patients inflicted with gastritis (Marshall et al, Med. J. Aust., 142(8): 439-44, 1985). Since these initial investigations, *H. pylori* and a recently discovered Helicobacter strain, *Helicobacter heilmanii*, have been shown to play an important role in causing such gastrointestinal disorders as peptic and gastric ulcers, gastric carcinoma, gastric lymphoma, gastritis, duodenitis, and esophagitis. In particular, these bacterium are estimated to have caused more than 90% of duodenal ulcers and up to 80% of gastric ulcers. Furthermore, *H. pylori* infected persons have a 2- to 6-fold increased risk of developing gastric cancer and mucosal-associated-lymphoid-type lymphoma.

[0004] Early detection of the presence of Helicobacter infection in the gastrointestinal tract improves an individual's prognosis. If detected early, *H. pylori* can be successfully treated with common antibiotics, such as penicillin or erythromycin, with no significant relapse in occurrence of disease. Moreover, early detection and immediate treatment by antibiotics is cost effective relative to other treatment regimes. For example, the cost of early treatment with antibiotics is a small fraction of the cost of surgery and post-surgical care. Thus, there is a great need for a reliable and simple method to diagnose the presence of *H. pylori* early in its infection cycle.

[0005] One method which has been employed for detecting the presence of *H. pylori* and disease conditions associated with it, requires the insertion of an endoscope into the stomach of a patient and withdrawal of a biopsy specimen for direct visual examination of the gastric mucosa tract. This method, however, is highly invasive, often causing significant patient discomfort, and requires trained personnel to carry out the procedure.

[0006] Another method for the detection of *H. pylori* infection requires collecting gas in the gastric cavity, and detecting in this gas ammonia and organic amines that are generated due to activities of the bacilli (see, e.g., U.S. Pat. No. 6,312,918). In this method, gas from the gastric cavity is led into the oral cavity by generating a vomiting-reflex, and the gas is collected by means of a metering suction pump which causes the gas to flow through a detection tube which changes color when ammonia and organic amines are present. Again, however, this technique is invasive, causes discomfort to the patient and is relatively expensive to perform.

[0007] Serological tests for *H. pylori* infection are somewhat less invasive. In these methods, a sample of blood is withdrawn and tested for the presence of IgA or IgG antibodies to *H. pylori* (see, e.g., U.S. Pat. No. 5,989,840). About twenty days from the time of infection, however, are required for antibodies against the bacterium to manifest themselves which can significantly compromise early detection. Also, antibodies can remain for 6-24 months after the bacteria have been eradicated, leading to a falsely positive result in about 10 to 15% of patients.

[0008] Breath tests have also been proposed for the detection of *H. pylori* infection. These tests exploit the fact that *H. pylori* produce and release urease, which catalyzes the degradation of urea into ammonia and carbon dioxide. For example, in one approach to a breath test (see, e.g., U.S. Pat. Nos. 4,830,010 and 6,067,989 and WO 97/26827), isotopically-labeled urea (e.g., 13C, 14C or 15N), in solid or liquid form, is orally ingested by the patient. If present, the bacteria convert the ingested urea to carbon dioxide and ammonia in the stomach. The concentration of isotopically labeled carbon, in the form of carbon dioxide, or nitrogen, in the form of ammonia, in a breath sample is then measured by mass spectrometry or a near infrared laser. Radioactive isotopes, however, have a relatively short half-life and raise safety and health issues for technicians as well as the patients. Non-radioactive isotopes possess other disadvantages; for example, the relative natural abundance of 13C is approximately 1% and thus, it is difficult to measure the amount of the isotope in a sample and the cost and complexity associated with the mass spectrometry pose significant drawbacks in any event.

[0009] In an alternative breath analysis method, a color changing indicator is used instead of a mass spectrometer or an infrared laser (see, e.g., WO97/30351). In this method, urea is administered to the subject and the subject's breath is thereafter analyzed for the presence of ammonia through the use of a composition which changes color when ammonia is present in the breath. Although it is disclosed that pH sensitive dyes may be used for this purpose, the preferred color indicator is a complex of a transition metal ion with the ammonia in an acidic environment. Complexes of the transition metal ions can be highly colored and can, therefore, form the basis of an indicator of the presence of ammonia. Disadvantageously, however, the metal ion, in addition to forming a complex with ammonia, also forms complexes with other substances present in the aqueous solution. These complexes also trigger a color change and therefore, can significantly bias the results of the test.

SUMMARY OF THE INVENTION

[0010] Among the several aspects of the invention therefore, is provided a method and apparatus for the detection of *H. pylori* and other bacteria associated with the catalytic degradation of urea to ammonia and carbon dioxide in the

gastrointestinal tract of a subject. Advantageously, the method is non-invasive, relatively inexpensive to perform, does not require the use of relatively expensive equipment, and does not provide false positives.

[0011] Briefly, therefore, one aspect of the present invention is a method for detecting in the gastrointestinal tract of a subject, the presence of a bacteria which when present in the gastrointestinal tract of the subject is associated with the catalytic degradation of urea to ammonia and carbon dioxide. The method comprises delivering a source of urea to the gastrointestinal tract of the subject, obtaining a fluid sample from the subject after the delivery of the urea source, contacting the fluid sample with a sensor, and optically detecting a color change in the sensor (which is indicative of the presence of ammonia in the fluid sample). The sensor comprises a polymeric material and a dye associated with the polymeric material, the dye having the capacity to become deprotonated and undergo a color change in the presence of ammonia. The contact conditions are controlled so that the sensor responds to the presence of ammonia in the fluid sample but not to the pH of the fluid sample by undergoing an optically discernible color change.

[0012] The present invention is further directed to such a method in which the fluid sample is combined with an aqueous solution to allow any ammonia in the fluid sample to dissolve into the aqueous solution. The aqueous solution is then contacted with a sensor which comprises a porous, hydrophobic polymer having a dye embedded within its pores. The dye has the capacity to be deprotonated and undergo a color change in the presence of ammonia gas. When the aqueous solution is contacted with the polymer under conditions which enable the pores of the polymer to be permeated by gaseous ammonia derived from the fluid sample, therefore, a color change in the dye is optically detected.

[0013] A further aspect of the invention is a device for carrying out the breath test. The device may be employed to detect the presence of a bacteria in the gastrointestinal tract of a subject associated with the catalytic breakdown of urea to carbon dioxide and ammonia. In general, the device comprises a breath sampler including a disposable breath handler and a detection unit. The breath handler is valved to permit inhalation there through which bypasses the detection unit, but exhalation is directed through the detection unit. The presence of ammonia in the exhalation is sensed by an ammonia sensing membrane in the detection unit. In one embodiment, the detection unit includes a container carrying liquid in which the membrane is submersed. In another embodiment, the breath sampler is constructed for inhibiting acquisition of ammonia from sources within the subject's mouth.

BRIEF DESCRIPTION OF THE DRAWINGS

[0014] These and other features, aspects, and advantages of the present invention will become better understood with regard to the following description, appended claims and accompanying figures where:

[0015] **FIG. 1** is an elevation of a breath sampler illustrated in use by a subject;

[0016] **FIG. 2** is an elevation of the breath sampler of **FIG. 1** with parts broken away to show internal construction;

[0017] **FIG. 3** is a perspective of the breath sampler and an optical reader into which a portion of the breath sampler may be received;

[0018] **FIG. 4** is an elevation of a breath sampler and optical reader of a second embodiment held by the subject in use;

[0019] **FIG. 5** is the breath sampler of the second embodiment;

[0020] **FIG. 6** is an exploded perspective of the breath sampler and optical reader of the second embodiment together with a battery charger;

[0021] **FIG. 7** is a depiction of the total amount of ammonia and ammonium, NH4+3, detected by the sensors of the present invention versus the known amounts of ammonia and ammonium in the test solutions ranging from about 0.1 ppm to about 100 ppm as measured by a chemical analyzer (Cobas);

[0022] **FIG. 8** is a kit of the present invention;

[0023] **FIG. 9** is a breath sampling system including a breath sampler and optical reader of a third embodiment;

[0024] **FIG. 10** is an exploded perspective of the breath sampling system of **FIG. 9**;

[0025] **FIG. 11** is a top plan view of the breath sampler of **FIG. 9** illustrating a second position of a breath handler of the breath sampler in phantom; and

[0026] **FIG. 12** is a section of the breath sampler of **FIG. 9**.

[0027] Corresponding reference characters indicate corresponding parts throughout the several views of the drawings.

DETAILED DESCRIPTION OF THE INVENTION

[0028] Among the various aspects of the present invention, is the provision of various methods and apparatus for use in the detection of *H. pylori* and other bacteria which are associated with the catalytic breakdown of urea to carbon dioxide and ammonia in the gastrointestinal tract. In general, the method comprises administering urea to a subject, obtaining a fluid sample from the subject after the administration of the urea, contacting the fluid sample with a sensor which undergoes a color change in the presence of ammonia, and optically reading the sensor to detect ammonia in the fluid sample.

[0029] Advantageously, the method of the present invention provides a means to detect the presence of any bacteria associated with the degradation of urea to ammonia and carbon dioxide when colonized in the gastrointestinal tract of a subject. In one embodiment, the subject is a mammal. In another embodiment, the subject is a livestock animal, zoo animal, a companion animal or a human. In a preferred embodiment, the subject is a human.

[0030] A number of bacterial strains possess urease associated activity and colonize within the gastrointestinal tract of a variety of subjects. For example, when the subject is a human, many species of Helicobacter, including *H. pylori, H. heilmanii* colonize within the gastrointestinal tract (Hirschl A. M., Wien Klin Worchenschr, 106(17): 538-42, 1994). Additionally, a coccoid organism, preliminarily suggested to

be related to Staphylococcus, has been cultivated from gastric biopsy specimens obtained from human subjects (Solnick and Schauer, Clinical Microbiology Reviews, 14(1): 59-97, 2001). Moreover, when the subject is a primate, *H. nemestinae* has been show to colonize within the gastrointestinal tract (Solnick and Schauer, Clinical Microbiology Reviews, 14(1): 59-97, 2001). Further, when the subjects are felines or canines, many distinct species of Helicobacter including *H. bizzozeronii, H. salomonis* and *H. felis* colonize within the gastrointestinal tract (Solnick and Schauer, Clinical Microbiology Reviews, 14(1): 59-97, 2001).

[0031] The bacteria to be detected, accordingly, will vary greatly depending upon the particular subject being examined. In one embodiment, the subject is a human and the bacteria to be detected is a Helicobacter. In this embodiment, for example, the bacteria to be detected may be *H. heilmanii* or *H. pylori*. Currently, the detection of *H. pylori* in human subjects is of particular clinical importance.

[0032] *H. pylori* and other bacteria possessing urease associated activity that colonize in the gastrointestinal tract of a subject, as described above, have been shown to cause a number of gastrointestinal disorders. These disorders include gastritis, peptic ulceration, gastric cancer, non-ulcer dyspepsia, duodenal ulcers, gastric ulcers, duodenitis, gastric non-Hodgkin's lymphomas, intestinal metaplasia, adenocarcinoma, and esophagitis. Accordingly, another aspect of the present invention is a method to diagnose gastrointestinal disorders caused by the bacteria described above. The methods of the invention may be employed to detect any stage of bacterial infection including early or late stages. Preferably, however, diagnosis is made during the early stages of infection before significant damage is done to the gastrointestinal tract of the subject.

[0033] Bacteria having urease associated activity that colonize in the gastrointestinal tract of a subject, as described above, have also been linked to disorders associated with the liver. In particular, *H. pylori* and *H. heilmanii* have been associated with hepatitis (McCathey et al, Helicobacter, 4(4): 249-59, 1999). Accordingly, another aspect of the present invention is a method for the diagnosis of liver disorders caused by the bacteria described above. Preferably, diagnosis is made during the early stages of infection before significant damage is done to the liver tissue.

[0034] In each of these methods, it is preferred that substantially all substances present in the gastrointestinal tract of the subject that either may lead to the production of ammonia, such as a food source, or may impact the urease associated activity of the bacteria, are eliminated prior to administration of the urea source to the subject. Thus, for example, the subject preferably fasts for at least about 4 hours, typically about 4 to about 24 hours prior to administration of the source of urea and collection of the fluid sample. More preferably, the subject fasts for about 6 to about 12 hours prior to administration of the source of urea collection of the fluid sample.

[0035] Bacteria possessing urease associated activity may reside in the oral cavity. When the fluid sample to be obtained from a subject is a breath sample, therefore, the subject may optionally be given a mouth wash comprising an antibacterial agent prior to administration of the source of urea. The mouth wash will thus tend to decrease the population of such bacteria in the oral cavity and thereby reduce potential bias in the sample collection process.

[0036] In general, any source of urea, in solid or liquid form may be administered to the subject. Such sources include any composition that may be converted to urea in vivo or otherwise serve as a substrate for the urease associated activity of the bacteria to be detected. By way of example, the urea source may constitute urea, per se, or it may be a derivative of urea. In one embodiment, the urea source is carbonyldioxide (H2NCONH2) which is commercially available from a variety of sources such as Sigma-Aldrich (Saint Louis, Mo.).

[0037] The amount of the urea source administered to the subject is preferably sufficient to produce a detectable concentration of ammonia in a fluid sample taken from a subject, without undue adverse side effects to the subject, such as toxicity, irritation or allergic responses. For a particular subject, the amount may vary and generally depends upon a variety of factors such as the form of the urea source, the particular fluid sample to be used, the weight of the subject, and species of the subject. In general, however, the amount administered will be from about 1 milligram to about 20 milligrams of urea per kilogram body weight of the subject.

[0038] The urea source may be administered to the gastrointestinal tract of the subject by any generally known method. In one embodiment, administration is by oral ingestion of urea, in single or multiple doses. The particular dosage form used to administer the urea may be, for example, in solid tablets or capsules, or in liquid solutions or emulsions. Moreover, urea may be administered essentially in pure form, as detailed above, or as part of a composition. Compositions useful in administration of urea may also contain pharmaceutically-acceptable components such as, for example, diluents, emulsifiers, binders, lubricants, glydants, colorants, flavors and sweeteners. Suitable components included in the composition, however, preferably do not interfere with hydrolysis of the urea, or generate appreciable quantities of carbon dioxide or ammonia in the gastrointestinal tract. A preferred optional component is one which delays gastric emptying, thereby increasing the length of time that the administered urea is present in the stomach.

[0039] After administration of the urea source to the subject, a period of time sufficient for the bacteria to catalyze urea to ammonia and carbon dioxide is allowed to elapse before collection of the fluid sample. In one embodiment, about 1 to about 120 minutes elapse after administration of urea prior to collection of the fluid sample. In another embodiment, about 5 to about 60 minutes elapse after administration of urea prior to collection of the fluid sample. In still another embodiment, about 10 to about 30 minutes elapse after administration of urea prior to collection of the fluid sample.

[0040] After a suitable period has elapsed, a fluid sample is obtained from the subject. The fluid sample may be any fluid, gaseous or liquid, containing a detectable amount of ammonia gas resulting from the urease associated activity of the bacteria to be detected. Suitable fluid samples include a breath sample, a saliva sample, a perspiration vapor sample, a gastric reflux sample and a tear sample. The volume of the fluid sample collected will depend, in part, upon the fluid type and source and the sensitivity of the sensor employed and can readily be determined by a skilled artisan.

[0041] In one embodiment, the fluid sample is a breath sample obtained from the subject's lungs through the nose, mouth, trachea, or other external orifice of the subject. Typically, and most conveniently the breath sample may be collected by having the subject exhale (or blow) into a gas collection apparatus. For example, the subject may exhale into a balloon and the contents of the balloon may be directly or indirectly transferred to a sensor for analysis. Alternatively, and more preferably, the subject exhales directly into a sensor apparatus of the type depicted in any of the various figures appearing and described in greater detail elsewhere herein.

[0042] In another embodiment, the fluid sample is a saliva or tear sample. A saliva sample may be collected by any means generally known in the art. For example, the saliva sample may be collected by having the subject expectorate into the collection device. If the subject has difficulty doing this, substances may be contacted with the buccal cavity to generate a reflex stimulation of saliva by the saliva glands. These substances illustratively include citric acid or milk. Alternatively, the saliva or tear sample may be collected by a sample probe. The sample probe may comprise a swab on a support stick which is placed into the mouth of a subject to collect saliva or the eye of the subject to collect a tear which is then transferred from the swab to the collecting apparatus.

[0043] In still another embodiment, the fluid sample is a perspiration vapor sample. The perspiration vapor sample may be collected by any means generally known in the art. For example, the perspiration vapor sample may be collected using a dermal patch device which is placed directly on the skin of the subject. Under the influence of the subject's body heat, which is readily conducted from the surface of the skin through the liquid phase, the liquid water component of the perspiration will tend to evaporate. Such volatilized water can thereby pass through the gas permeable filter and leave the patch device. The device can further contain a microbead layer, where microbeads can desirably attach to the desired ammonia, thereby preventing it from escaping as a vapor through the gas permeable filter. In one embodiment, the trapped sample is then placed into the collecting apparatus and the presence of ammonia gas is then determined as described herein. In an alternative embodiment, the patch employed to collect the perspiration vapor sample has a dye embedded within its pores that changes color in response to the presence of ammonia gas. Accordingly, in this embodiment, the presence of ammonia gas in the perspiration vapor sample may be directly determined by observing a color change of the dye in the patch.

[0044] In yet another embodiment, the fluid sample is a gastric reflux sample. The gastric reflux sample may be collected by any means generally known in the art. For example, the gastric reflux sample may be collected by stimulating the throat or larynx producing vomiting-reflexive belching, called "eructation." A round-shaped structure at its tip can be used to stimulate the throat or larynx, preventing the inside of the oral cavity from scratching. The gastric gas is thereby directed to the oral cavity and into the measuring device.

[0045] Regardless of the nature of the fluid sample or the means for its collection, a sensor is used to detect the presence of ammonia in the fluid sample. In one embodiment, the fluid sample (whether gas or liquid) is directly contacted with the sensor. In another embodiment, the fluid sample (whether gas or liquid) is first combined with an aqueous solution to allow ammonia in the fluid sample to dissolve into the aqueous solution and the aqueous solution is then contacted with the sensor.

[0046] In those embodiments in which the fluid sample is first combined with an aqueous solution, the aqueous solution, in theory, may contain any composition which does not interfere with the detection of ammonia. For example, the aqueous solution preferably does not contain any compositions which react or otherwise deleteriously interact with ammonia (or ammonium) in the fluid sample. By way of illustration, the aqueous solution preferably does not contain compounds that covalently bind to or that form complexes with ammonia. In one embodiment, the aqueous solution comprises sterile water which has been adjusted to a desired pH. Ammonia (or ammonium) present in the fluid sample will readily dissolve in such a solution and, as described elsewhere, the ammonia can thereafter be detected using an ammonia sensor.

[0047] In an aqueous solution, ammonia may exist in an ionic (NH_4^+) or non-ionic (NH_3) form and the pH of the solution dictates which of these forms is predominant. For example, NH_4^+ is predominant in acidic solutions while NH_3 is predominant in basic solutions. Because the sensor of the present invention is tuned to measure ammonia, however, it is preferred that the aqueous solution have a neutral or basic pH. In one embodiment, the aqueous solution has a pH of 7 to about 9.5. In another embodiment, the aqueous solution has a pH from about 8.0 to about 9.0. Any suitable compound which does not interfere with the assay may be added to the aqueous solution in order to adjust the pH to a desired value. Exemplary compounds which may be used for this purpose include the hydroxides of alkali metals and alkaline earth metals, such as sodium hydroxide or potassium hydroxide.

[0048] To detect the presence of ammonia in the fluid sample, the fluid sample itself or an aqueous solution which has been combined or otherwise contacted with the fluid sample is brought into contact with an ammonia sensor. In general, the sensor is a polymer carrying a dye which is capable of being deprotonated and undergoing a color change in the presence of ammonia. When the sensor comes into contact with ammonia, therefore, the dye undergoes an optically detectable color change which can be observed by a visual or other optical inspection of the sensor.

[0049] In one embodiment, the sensor comprises a porous, hydrophobic polymeric material and the dye is embedded within the pores but is substantially absent from the remainder of the exposed surface of the material. After a fluid sample, e.g., a breath sample, is combined or otherwise contacted with an aqueous solution for a period sufficient for ammonia (ammonium ions) to dissolve into the solution, the aqueous solution is brought into contact with the sensor. Because the dye is substantially absent from the surface of the sensor, the exposed surface of the sensor will not undergo a significant color change. Ammonia dissolved in the aqueous solution, however, can permeate the pores of the sensor and deprotonate the dye to effect a discernible color change. Significantly, the pore size and hydrophobic character of the sensor combine to effectively exclude liquid

from the pores of the sensor. For all practical purposes, therefore, the pores of the sensor are impermeable to the aqueous solution, and as a result, the sensor will undergo a discernible color change in response to the presence of ammonia (ammonium ions) in the fluid sample but not in response to the pH of the aqueous solution. Stated another way, the sensor does not respond to the pH of the fluid sample but rather, to the presence of ammonia in the fluid sample independent of the pH of the fluid sample. Any significant color change by the sensor, therefore, is a positive indication that the pores have been permeated by ammonia.

[0050] In general, the degree of hydrophilicity or hydrophobicity can be determined by reference to the contact angle of a droplet of water placed on the surface of the polymer. As used herein, a polymer is considered to be hydrophilic if the contact angle is less than 30 degrees; conversely, a surface is considered to be hydrophobic if the contact angle of a drop of water placed on the surface is greater than about 100 degrees. (M. Cheryan, Ultrafiltration and Microfiltration Handbook **245-46** Technomic Publishing Co.). More preferably, the a surface is considered to be hydrophobic if the contact angle is between 100 and 150 degrees. Even more preferably, a surface is considered to be hydrophobic if the contact angle is 110 degrees.

[0051] As previously mentioned, the hydrophobicity of the sensor material, combined with the porosity of the sensor material can be controlled to substantially prevent aqueous solutions from permeating the pores of the sensor material. In one embodiment, the pore size is about 9.0 micrometers or less, preferably from about 3.5 microns to about 0.2 microns, more preferably about 2.5 microns or less as determined by bubble point pressure definition. In another embodiment, the average pore size of the polymer can range from about 2.5 microns to about 1 micron, preferably from about 2.0 microns to about 1.6 microns. Of course, it will be apparent to those skilled in the art that it is possible, and perhaps desirable, that the polymers can be made to include any variety of different and suitable pore sizes.

[0052] In an alternative embodiment when the fluid sample is a vapor, such as a breath sample, the fluid sample may be directly contacted with a hydrophobic polymer. Because the sensor is not coming into contact with a liquid solution in this embodiment, there is a reduced risk that a species other than ammonia gas is responsible for a color change in the sensor. Accordingly, the dye may be, but need not be contained substantially exclusively in the pores of the sensor material (i.e., the dye may be carried on the remaining exposed surface of the sensor) in this embodiment.

[0053] In accordance with the method of the present invention, a color change in the sensor reflects the presence of ammonia and not merely the pH of an aqueous solution which is in contact with the sensor. This color change is detected optically. In one embodiment, the color change is detected visually by the observation of the subject or a technician assisting the subject in the assay. In this embodiment, the color change may merely be read to confirm the presence or absence (but not the concentration) of ammonia in the fluid sample; alternatively, the degree of color change may be used as a quantitative or semi-quantitative measure of the concentration of ammonia in the fluid sample. In another embodiment, an optical reader is used to monitor the color change; for example, the optical reader may be a calorimetric reader, such as a spectrophotometer or laser, as more completely described in U.S. patent application Ser. No. 10/024,170 entitled "Ammonia and Ammonium Sensors," the entire content of which is hereby incorporated by reference (**FIG. 8**). The color change may merely be read by the optical reader to confirm the presence or absence (but not the concentration) of ammonia in the fluid sample; alternatively, the degree of color change may be used as a quantitative or semi-quantitative measure of the concentration of ammonia in the fluid sample.

[0054] In one embodiment, the color change is reversible. In this manner, the presence (or amount) of ammonia in a fluid sample or aqueous solution may be optically detected by observing a color change in the dye as a function of time.

[0055] The sensor may comprise any of a variety of polymeric materials. For example, the sensor may consist of polypropylene, polytetrafluoroethylene ("PTFE"), polyvinylidene difluoride ("PVDF"), fluorinated ethylene propylene polymers ("FEP"), acrylic-based polymeric compounds, acrylic-based fluorinate polymers, polycarbonate, polypropylene, polyvanilidine chloride, dimethyl polysiloxane and copolymers thereof, or combinations thereof.

[0056] The sensor may also assume any of a variety of geometric shapes. For example, the sensor may comprise regular or irregularly shaped particles, e.g., beads, relatively thin layers (supported or unsupported by other materials), or any of a wide variety of shapes which may be useful for optical readers or mere observation. In one embodiment, the polymer is in the form of a membrane.

[0057] In one embodiment, the dye is intimately embedded or bound within the porous structure of the polymer such that a negligible amount, if any, dye leaches from the polymer when the polymer is exposed to the aqueous solution. In general, any method known in the art may be employed to embed or bind the dye to the pores of the polymer, including those methods described in U.S. patent application Ser. No. 10/024,670 entitled "Hydrophobic Ammonia Sensing Membrane," which is hereby incorporated by reference in its entirety (**FIG. 9**).

[0058] In one embodiment of the present invention, the dye is associated with the polymer in a casting process. During casting, the polymer and the dye are formed into a casting solution containing a suitable solvent. Any suitable amounts of the polymer and dye can be blended or mixed into the casting solution. Typically, the casting solution includes at least about 0.1% by weight of the dye in a solution containing about 14% to about 24% by weight of polymer, and more preferably about 19% to about 21% by weight of polymer. The casting solution is then poured onto substrate, such as a mesh material, or glass substrate, and further processed by immersing the casting solution in an acidic solution under suitable conditions such that a precipitate is formed. In an embodiment, the acidic solution used during precipitation contains a suitable amount of methanol, including about 50% to about 100% by weight of methanol, preferably about 90% or more by weight, and has a pH of about 3 to about 4, and more preferably, about 3. The precipitate is then further washed and dried under suitable conditions to form the polymer with the dye embedded within the pores of the polymer. In an embodiment, the processed polymer can be dried at temperature ranging from about room temperature to about 100° C., and preferably, at

about 60° C. An example of the casting procedure illustrative of an embodiment of the present invention is detailed below in Example 1.

[0059] In another method, the dye is associated with the polymer by dip coating. During dip coating, a polymer preformed to the desired shape, such as a membrane, is immersed in an aqueous coating solution containing a dye and a solvent. In an embodiment, the solvent includes isopropyl alcohol, acetone, mixtures thereof and other suitable solvent materials. The coating solution can include any suitable amount of the dye and the solvent. In an embodiment, the coating solution includes at least about 0.05% by weight of the dye, more preferably, at least 0.2% by weight of the dye, in an aqueous solution containing about 10% to about 50%, and more preferably, about 30% by volume of the solvent. An example of the dip coating process illustrative of an embodiment of the present invention is discussed below in Example 2.

[0060] Any dye that is sensitive to and responds to changes in the amount of ammonia that permeates the pores of the polymer may be employed. In a preferred embodiment, the dye is a pH sensitive dye that becomes deprotonated and undergoes a color change in the presence of ammonia gas. Suitable pH sensitive dyes include bromophenol blue, bromothymol blue, methyl yellow, methyl orange, 2,4-dinitrophenol, 2,6-dinitrophenol, phenol red, cresol red and any mixtures thereof. Preferably, the dye selected is capable of reverting back to its original color when not contacted by ammonia gas. In addition, the dye selected is also capable of imparting to the sensor the ability to undergo a degree of color change which is directly proportional to the amount of ammonia gas that permeates into the polymer.

[0061] In one embodiment of the invention, a fluid sample is taken from the subject prior to administration of the urea source and the ammonia concentration is determined in accordance with the steps detailed above. The subject is then administered a urea source, as detailed above, and a number of fluid samples are then collected from the subject. The presence or concentration of ammonia gas in each sample is independently determined. Typically, about 1 to about 20 fluid samples are collected and quantified. Even more preferably, about 5 to about 10 fluid samples are collected.

[0062] The collection and testing of a fluid sample prior to the administration of urea, and one to several fluid samples after the administration of urea, is particularly advantageous to increase the accuracy of the method. By way of illustration, for example, prior to administration of urea, only a nominal amount of ammonia gas will be present in the fluid sample, e.g. on the order of less than about 1 part per million. After the administration of urea, however, the amount of ammonia gas present in the fluid sample will increase by a magnitude of about 10 to about 1000 fold. Accordingly, by comparing the amount of ammonia gas present in both pre and post urea fluid samples, the method of the present invention provides an extremely accurate means to determine the presence of a bacteria possessing urease associated activity in the gastrointestinal tract of a subject.

[0063] Although the method of the present invention may be carried out in a variety of apparatus, it is preferably carried out in a first embodiment using a breath sampler, generally indicated at **101** in **FIG. 1**. The breath sampler is shown in **FIG. 1** in use by a subject to obtain a breath sample for indication of the presence of *H. pylori* infection in the subject's gastrointestinal tract. The breath sampler **101** comprises a breath handler and a detection unit, indicated in their entireties by reference numerals **103** and **105**, respectively. The detection unit **105** is connected to the breath handler **103** for receiving the subject's breath sample, which may consist of one, but is usually several exhaled breaths. Generally, the breath sampler **101** is configured to permit inhalation through the breath handler **103** and exhalation through the detection unit **105**. The breath handler **103** includes an elongate tube **107** and a sample collection branch **109** formed in the illustrated embodiment as one piece with the breath handler from a suitable material. Referring now also to **FIG. 2**, the tube **107** is open at both a mouthpiece end **111** and an intake end **113** so that air may pass through the tube from the intake end through the open mouthpiece end. A check valve **115** in near the intake end **113** of the tube **107** restricts flow through the tube at the intake end to a direction toward the mouthpiece end **111** and blocks flow out of the intake end. In other words, the check valve **115** permits air to be taken in through the intake end **113**, but does not permit the subject's breath to pass out through the intake end. The check valve **115** may be of any suitable construction, such as a generally cone-shaped diaphragm having a slit in its small end. Air passing in from the intake end **113** passes through a larger base of the diaphragm and forces open the slit to pass through the check valve **115**. However, the subject's exhalation bears against the generally conical walls of the exterior of the diaphragm of the check valve **115** and pushes the walls inward toward the center, holding the slit in a closed position so that the flow of exhalation out through the intake end **113** is blocked.

[0064] The breath sample from the subject is directed downward into the sample collection branch **109** and into a container **117** of the detection unit **105**. The container **117** has a vent **119** which permits excess gas in the container to be exhausted to the atmosphere so that exhalation may flow into the container. A check valve **121** in the vent **119** permits flow of air out of the container **117** upon exhalation, but when the subject is not providing air pressure, closes the container vent to the influx of ambient air and to outflow of liquid L. Another check valve **123** disposed in the collection branch **109** permits the breath sample to flow through the branch and into the container **117**, but inhibits withdrawal of gas or liquid L from the container upon inhalation. Moreover, the check valve **123** helps to keep the liquid L from spilling out of the container **117**. The construction and operation of the check valves **121**, **123** can be the same as check valve **115** described above, except that the orientation of the check valves **121**, **123** is reversed to permit the breath sample to flow outwardly from the mouthpiece end **111** through the tube **107**, sample collection branch **109**, container **117** and vent **119**, and to block air flow through the vent, sample collection branch, container and tube toward the mouthpiece end. The check valves **115**, **121**, **124** are desirably biased to a closed position in the absence of a pressure differential across the valve so that the container **117** is normally substantially isolated from the breath handler **103** and the ambient air. It is to be understood that while the check valves **115**, **121** provide certain advantages and conveniences in use, they may be omitted without departing from the scope of the present invention. For example, the intake end of the tube may be closed off (not shown). Generally speaking, the breath handler **103**, detection unit

105, container 117 and check valves 115, 121, 123 are made of a medical grade plastic capable of being initially sterilized. However, the breath sampler 101 is preferably disposable so that an inexpensive plastic is desirably employed.

[0065] In the embodiment of FIG. 1, the container 117 of the detection unit 105 is constructed for holding a volume of liquid L, such as sterilized water, and the lower end of the sample connection branch 109 which includes a diffuser head 125 is immersed in the liquid. Exhalation passes out of the sample collection branch 109, through small openings 126 in the diffuser head 125 and into the liquid L. Diffusion of the breath sample by the head 125 facilitates retention of any ammonia in the sample by the liquid L. An ammonia sensing membrane 127 is located on the bottom of the container 117 in opposed relation with the diffuser head 125 so that the breath sample leaving the collection branch is spread within the liquid L and over the membrane. The membrane 127 is of the type which detects the presence of ammonia (and ammonium) in the breath sample and indicates the presence of ammonia through a change of color. Examples of suitable ammonia sensing membranes are described in co-assigned U.S. patent application Ser. No. 10/024,170, entitled Ammonia and Ammonium Sensors, and U.S. patent application Ser. No. 10/024,670, entitled Hydrophobic Ammonia Sensing Membrane, previously incorporated herein by reference. The membrane 127 is attached in a suitable manner, such as by ultrasonic or thermal welding to a bottom wall of the container 117.

[0066] An optical reader, indicated generally at 131 in FIG. 3, has an opening 133 in an upper surface for receiving at least the lower end of the container 117. In the illustrated embodiment, the container 117 is formed of an optically clear material so that the membrane 127 can be examined by the optical reader 131 through a wall of the container. The optical reader 131 may be of the type which sends light signals toward the membrane 127 so that a photo-detector (not shown) may read the color of the membrane. The optical reader 131 has a display 137 to output a suitable message indicative of the presence or absence of ammonia in the sampled breath based on the color of the membrane 127 detected. A suitable optical reader is disclosed in the aforementioned U.S. patent application Ser. Nos. 10/024,170 and 10/024,670.

[0067] In use, the subject places the mouthpiece end 111 of the breath handler tube 107 in the mouth and seals around the tube with the lips. Typically, the nasal passages are occluded, such as by placing a clip (not shown) on the nose, so that the subject breathes only through the breath sampler 101. Previously, the subject will have blown a baseline reading through the breath sampler 101 which is read by the optical reader 131. The subject will have subsequently been prepared and have ingested urea so that ammonia may be generated in the presence of *H. pylori* in the gastrointestinal tract. The subject then draws air into the lungs by inhaling. The check valves 121, 123 prevent liquid L or any gas from the container 117 from being aspirated into the subject's lungs by blocking flow toward the mouthpiece end 111. However, air passes freely into the intake end 113 and through the check valve 115. The subject then exhales, and breath passes into the tube 107 through the mouthpiece end 111 where it is blocked by the check valve 115 at the intake end 113, but may pass through the check valve 123 in the sample collection branch 109 into the container 117 and thence out of the container through the check valve 121 and vent 119. The breath sample passes into the liquid L and over the ammonia sensing membrane 127 which changes color if ammonia (or ammonium) is present in a sufficient quantity in the breath sample. Preferably, the subject should breathe in and out several times to provide sufficiently large sample to the ammonia sensing membrane 127. The particular construction of the breath sampler 101 shown and described herein makes it much more convenient for the subject to inhale and exhale multiple times without unsealing the lips from the breath handler tube 107 or aspirating liquid from the container 117.

[0068] Referring now to FIGS. 4-6, a breath sampler and optical reader of a second embodiment are generally designated at 201 and 231, respectively (collectively, "a breath sampling system"). The breath sampler 201 comprises a breath handler 203 and a detection unit 205. Corresponding parts of the breath sampler 201 and optical reader 231 will be indicated by the same reference numerals as the breath sampler 101 and optical reader 131 of the first embodiment, plus 100. The construction of the breath handler 203 may be substantially the same as the breath handler 103 of the first embodiment. However, the detection unit 205 is a much flatter container 217 defining a shallow internal volume through which air passes to a vent 219 and the container does not hold any liquid. A check valve 221 is located at the vent 219 and a check valve 223 is located in the collection branch 209, as with the first embodiment. The diffuser head 225 has its lower surface disposed just above the ammonia sensing membrane 227 so that the breath sample passes out of dispersed openings 226 and is spread over and contacts the membrane. In this embodiment, the ammonia sensing membrane 227 is operable to detect ammonia directly from the breath sample without passage into a liquid.

[0069] It is to be understood that liquid may be used in the container 217, or for that matter not used in the container 117 of the first embodiment, without departing from the scope of the present invention. Where liquid is employed, the container is likely, but not necessarily larger in volume. Liquid has an advantage in that it accumulates ammonia in multiple exhalations from the subject, making it easier for the ammonia sensing membrane (127, 227) to detect the minute amounts of ammonia in the breath sample. If liquid is not used, as in the embodiment illustrated in FIGS. 4-6, it may be desirable to provide a cap 229 (FIG. 5) which can be fitted over the mouthpiece end 211 of the breath handler 203 to assist sealing the sample within the breath sampler 201. It is envisioned that the cap 229 might be used instead of the check valve 223 in a more inexpensive version of the breath sampler 201, or omitted when the check valve 223 is present.

[0070] The smaller, portable optical reader 231 includes housing having a reader portion 241, an output display 237, and a handle 243 which houses a rechargeable battery (not shown). The battery allows the optical reader 231 to be self-contained, i.e., operable to provide a reading remote from any other power source. The breath sampler 201 is preferably disposable and constructed for releasable, snap together attachment to the optical reader 231. However, other forms of attachment of the breath sampler 201 to the optical reader 231 may be employed. The optical reader 231 is light weight and portable so that it can be attached to the breath sampler 201 as it is being used by the subject for an immediate indication of an *H. pylori* infection in the gas-

trointestinal tract. As shown in **FIG. 4**, the subject may hold the handle **243** of the optical reader **231** while breathing through the breath sampler **201** mounted on the optical reader. A battery charger **245** is provided for recharging the battery housed in the handle **243** of the optical reader **231** (**FIG. 6**).

[**0071**] A further aspect of the invention provides a kit to detect the presence of a bacteria capable of catalyzing urea to carbon dioxide and ammonia. The kit comprises a sterile disposable breath sampler (e.g., breath sampler **201**), a bottle **251** which contains urea concentrate, which can have a different volume with variant concentrations of urea, or a tablet **253** with equivalent solid urea (USP), and a nasal cannula **255**.

[**0072**] In one embodiment, the subject will drink the urea concentrate from the bottle **251** (or take the tablet **253**) and then apply the nasal cannula **255** to occlude the nasal passages. After the appropriate amount of time has gone by, the subject breathes through the breath sampler **201** for a breath ammonia measurement at designated time intervals. The optical reader **231** will display the measured ammonia with a present mathematical model to diagnose if the subject has been infected by bacteria that can catalyze urea into ammonia and carbon dioxide.

[**0073**] In one embodiment, the subject will drink the urea concentrate and then put on the nasal cannula to breath through the breath sampler for a breath ammonia measurement at designated time intervals. The optical reader will display the measured ammonia with a present mathematical model to diagnose if the subject has been infected by bacteria that can catalyze urea into ammonia and carbon dioxide.

[**0074**] A breath sampler and optical reader, generally designated at **301** and **331**, respectively, are shown in FIGS. **9-12**. The breath sampler **301** comprises a breath handler **303** and a detection unit **305**. Corresponding parts of the breath sampler **301** and optical reader **331** will be indicated by the same reference numerals as breath sampler **101** and optical reader **131** of the first embodiment, plus **200**. The breath handler **301** has a construction similar to that of the breath handler **101**, including a tube **307** and a sample collection branch **309**. The tube **307** has a mouthpiece end portion **311** and an intake end portion **313** so that air may pass through the tube from an air intake **313A** to a breath sample opening **311A** in the distal end of the mouthpiece end portion (see **FIG. 12**). A check valve **315** located in the air intake **313A** of the tube **307** restricts flow through the tube at the air intake end portion **313** to a direction toward the mouthpiece end portion **311** and blocks flow out of the tube through the air intake. The check valve **315** may be of any suitable construction, and is illustrated in FIGS. **9-12** as a disk including a rigid, perforated substrate **315A** and a flexible diaphragm **315B** having a central opening. Upon inhalation, the pressure drop behind the diaphragm **315B** pulls it off of the substrate **315A**, allowing air to flow in through the holes in the substrate and through the central opening of the diaphragm. Upon exhalation, positive air pressure behind the diaphragm **315B** (and the resiliency of the diaphragm) urges the diaphragm against the substrate, covering the holes in the substrate and central hole in the diaphragm to block the flow of air past the check valve **315** out of the tube **307**. A check valve **321** is located at a vent **319** in a collection container **317** of the detection unit **305**, and a check valve **323** is located in the collection branch **309**, as with the first embodiment. These check valves (**321**, **323**) are of the same construction as the check valve **315** and are oriented to permit the flow of air as described above for the breath sampler **101** of the first embodiment. A greater or lesser number of check valves (e.g., valves **315**, **321**, **323**) may be used without departing from the scope of the present invention. For example and without limitation, the check valve **321** at the vent **319** might be omitted. A lower end of the collection branch **309** includes a breath sample outlet **310** which opens into liquid L covering the membrane **327** (**FIG. 12**). It will be understood that the breath sampler **301** may operate without the liquid L and not depart from the scope of the present invention.

[**0075**] The mouthpiece portion **311** of the breath handler **303** of the third embodiment is tapered in both a width and height dimension toward the breath sample opening **311A** at the end of the mouthpiece portion. It will be understood that the tapering may occur in only one of the dimensions without departing from the scope of the present invention. The tapering facilitates reception of the mouthpiece portion **311** into the mouth (see **FIG. 9**). Moreover, the length of the mouthpiece portion **311** is selected so that the breath sample opening **311A** may be placed far back in the mouth, essentially at the throat. This placement tends to avoid exhaled air passing into the mouth significantly before it passes into the breath sample opening **311A**. It is possible for the mouth to contain substances which can generate ammonia, which could give a false reading. The configuration of the breath handler **303** helps the breath sampler **301** to avoid collection of ammonia from this source.

[**0076**] An optical reader **331** includes a housing having a reader portion **341**, an output display **337**, and a handle **343** which encloses a rechargeable battery (not shown). The battery allows the optical reader **331** to be self-contained, so that the subject may use the breath sampling system (breath sampler **301** and optical reader **331**) apart from any fixed power source, as shown in **FIG. 9**. The subject holds the handle **343** of the optical reacher **331** and uses the optical reader to position the breath sampler **301** for taking the mouthpiece portion **311** into the mouth. The breath sampler **301** is preferably disposable and capable of releasable snap-together connection with the optical reader **331**, but other configurations are possible within the scope of the present invention. As with the breath sampling system of the second embodiment, the subject may receive an immediate indication from the portable optical reader **331** of the presence or absence of *H. pylori* in the gastrointestinal tract when the test is conducted. A battery charger **345** is provided for recharging the battery housed in the handle **343** (**FIG. 10**).

[**0077**] The collection branch **309** is received in the collection container **317** through an opening **317A** and is pivotable in the opening with respect to the collection container about the axis of the collection branch. It is preferable to have the air intake **313A** of the breath handler **303** offset from (i.e., not located directly above) the vent **319** so that as the subject breathes in and out several times through the breath handler **303**, the subject does not inhale the material expelled from the collection container **317** through the vent. **FIG. 11** illustrates two positions of the breath handler **303** with respect to the collection container

317. These two positions may be achieved by turning the breath handler 303 within the collection container 317. Thus, when the subject grasps the handle 343 of the optical reader 331 with either hand, the breath handler 303 may be turned relative to the collection container 317 to offset the air intake 313A of the breath handler from the vent 319 while allowing the optical reader to be held in a comfortable position for receiving the mouthpiece end portion 311 into the mouth. It is to be understood that the offset may be achieved in other ways (e.g., without pivoting of the breath handler) without departing from the scope of the present invention.

[0078] The detailed description set-forth above is provided to aid those skilled in the art in practicing the present invention. Even so, this detailed description should not be construed to unduly limit the present invention as modifications and variation in the embodiments discussed herein can be made by those of ordinary skill in the art without departing from the spirit or scope of the present inventive discovery.

[0079] All publications, patents, patent applications and other references cited in this application are herein incorporated by reference in their entirety as if each individual publication, patent, patent application or other reference were specifically and individually indicated to be incorporated by reference.

[0080] Without further elaboration, it is believed that one skilled in the art can, using the preceding description, utilize the present invention to its fullest extent. The following preferred specific embodiments are, therefore, to be construed as merely illustrative, and not imitative of the remainder of the disclosure in any way whatsoever.

EXAMPLES

Example 1

[0081] Construction of the polymer with a dye embedded in the pores by casting

[0082] A casting solution was prepared by blending a PVDF solution (e.g., hydrophobic membrane material) with bromophenol blue (pH sensitive dye). The casting solution included about 0.1% by weight of bromophenol blue in a solution containing about 20% by weight of PVDF. The PVDF solution was made with a suitable solvent including, for example, dimethyl acetamide, dimethyl formamide, triethyl phosphate, dimethyl sulfoxide or the like.

[0083] A methanol bath solution was prepared by mixing methanol with varying amounts of 0.1 N hydrochloric acid ("HCl") to adjust the acid bath solution. The pH of the methanol bath solution ranged from about 3 to about 4. An acid washing solution was separately prepared with 0.1 N HCl having a pH of about 3.

[0084] A polyester support mesh passes through the casting solution continued in a V-shape dispensing device, sometime referred to as a V-box. The mesh exits and draws the casting solution through a slit on the V-box. The entire mesh structure becomes coated by and impregnated with the solution such that the casting solution is evenly spread on both sides of the mesh. The mesh substrate was a commercially available mesh material. Once exited, the casting solution was immersed in the methanol bath for about 3 minutes to about 18 minutes such that a precipitate formed. The precipitate was subsequently dried in air at about room temperature to about 80° C.

[0085] It should be appreciated that the above casting procedure can be suitably modified. In this regard, the dye can be added at any suitable stage during the casting procedure. For example, the dye can be added to the acid bath solution and then to the casting solution to form the precipitate. The casting procedure can also include additional washing of the precipitate prior to drying. The washing can be conducted with the acid wash solution as discussed above or other suitable washing media including water. Further, the drying stage can be conducted at room temperature or conducted under suitably higher temperatures in order to decrease the drying time. In addition, a variety of other suitable substrates in place of the mesh material can be utilized, such a glass substrate or a metal substrate.

Example 2

[0086] Construction of the polymer with a dye embedded in the pores by dip coating

[0087] A dip coating solution was prepared by mixing about 0.2% by weight bromophenol blue into an aqueous solution containing about 30% by volume of isopropyl alcohol.

[0088] A polymer was prepared that included about 19% to about 21% by weight of PVDF. The PVDF was immersed into the dip coating solution for about 2 to about 8 minutes and then air dried to form the ammonia sensing polymer. It should be appreciated that the dip coating process of the present invention can be modified in a variety of different and suitable ways.

Example 3

[0089] Physical Characteristics of the Sensors

[0090] A number of experiments were conducted to demonstrate the efficacy of the sensors of the present invention for detecting the presence of ammonia in a fluid sample. The sensors that were tested were prepared by procedures in accordance with an embodiment of the present invention as described above.

[0091] Porous Microstructure

[0092] The porous microstructure of the sensors made in accordance with an embodiment of the present invention were characterized employing a scanning electron microscopy technique. The test results demonstrated that the sensors of the present invention have a microporous sponge type structure with multilayered passageways in the mesh forming the pores. Dye is deposited on the internal surfaces of the mesh-like layers of the membrane.

[0093] pH Effects

[0094] The sensors of the present invention were tested to determine the effects of pH on the sensor's ability to detect ammonia. In an experiment, the sensors made in accordance with an embodiment of the present invention were placed in a 50 ml beaker containing 10 ml of distilled water and having a pH of about 7. The sensor, bromophenol blue, displayed no color change. After three minutes, an acid

buffer solution, comprised of 15% sodium citrate, 15% citric acid, and 70% water was added to the beaker until the pH was about 4.7. Again, the sensor displayed no color change. Lastly, 0.1M NaOH was gradually added until the pH of the solution was about 10. Again, the sensor displayed no color change.

[0095] This demonstrates that the sensor will not react with changes of pH from ranges of pH 4.7 to pH 10. Therefore, changes in the pH have negligible, if any, effects of the calorimetric reactivity of the ammonia sensor of the present invention without the presence of ammonia.

[0096] Ammonia Selectivity

[0097] The ammonia sensors made in accordance with the present invention were tested to demonstrate the detection capabilities with respect to the detection selectivity for ammonia in comparison to other materials. In this test example, the sensors, bromophenol blue, were tested to evaluate their detection selectivity with respect to ammonia in the presence of carbon dioxide or ammonium.

[0098] The ammonia sensor was placed into a 50 ml small beaker. 10 ml of 1000 ppm CO_2 solution was then added into the beaker. The sensor displayed no visible color change. After three minutes, the sensor still displayed no color change. An acid buffer solution comprising of 15% sodium citrate, 15% citric acid, and 70% water was added until the pH was about 4.7. The acid buffer solution created CO_2. Again, there was no color change. Next, one drop of 1000 ppm NH_4OH was added to the beaker; allowing NH4+ to be present in the beaker, but not NH3. The sensor displayed no color change. Further, 0.1M NaOH was gradually added to the beaker to increase the pH of the solution to 10. Gradually, the sensor's color changed from the original yellow to light blue, blue, dark blue, and then darker blue as more NH4+was converted to NH3. Finally, to show that the sensor color is reversible, an acid buffer solution described above was again added to lower the pH. The sensor color visibly changed back to the original yellow.

[0099] The test results indicated that the presence of carbon dioxide had negligible, if any, effects on the detection of ammonia. In this regard, the sensor did not detect the carbon dioxide or the ammonium and, thus, exhibited an enhanced selectivity with respect to the detection of ammonia. The amount of ammonia detected by the ammonia sensor of the present invention demonstrated an essentially linear correlation with respect to known amounts of ammonia as measured by Cobas. Further, the tests results showed that the color changing reaction in the presence of ammonia is fully reversible.

[0100] Detection Accuracy

[0101] The sensors of the present invention were tested to demonstrate the accuracy and sensitivity of the ammonia detection capabilities of the present invention. The test results showed that the ammonia sensor of the present invention accurately detected amounts of ammonia in a test solution ranging from about 0.01 ppm to about 800 ppm. The determination was based on a correlation (R2=0.8635) of the amount of ammonia detected by the sensors of the present invention versus the known amounts of ammonia in the test solutions ranging from about 0.1 ppm to about 100 ppm as measured by a chemical analyzer (e.g., Cobas Mira) as shown in **FIG. 7.**

What is claimed is:

1. A method for detecting in the gastrointestinal tract of a subject the presence of a bacteria which when present in the gastrointestinal tract of the subject is associated with the catalytic degradation of urea to ammonia and carbon dioxide, the method comprising:

(a) delivering a source of urea to the gastrointestinal tract of the subject,

(b) obtaining a fluid sample from the subject after the delivery of the urea source,

(c) combining the fluid sample with an aqueous solution,

(d) contacting the aqueous solution with a sensor after the aqueous solution has been combined with the fluid sample, the sensor comprising a porous hydrophobic polymer having a dye embedded within the pores but not on the exposed surface thereof, the dye being capable of being deprotonated and undergoing a color change in the presence of ammonia, the pores being permeable to ammonia gas derived from the aqueous solution but impermeable to the aqueous solution whereby ammonia gas derived from the aqueous solution can permeate the pores and deprotonate the dye to effect a color change, and

(e) detecting a color change in the sensor after the sensor is contacted with the fluid sample.

2. The method of claim 1 wherein the bacteria causes a gastrointestinal associated disorder in the subject, the gastrointestinal associated disorder being selected from the group consisting of gastritis, peptic ulceration, gastric cancer, non-ulcer dyspesia, duodenal ulcers, gastric ulcers, duodenitis, gastric non-Hodgkin's lumphomas, intestinal metaplasia, adenocarcinoma, and esophagitis.

3. The method of claim 1 wherein the bacteria is a Helicobacter.

4. The method of claim 1 wherein the bacteria is *Helicobacter pylori.*

5. The method of claim 1 wherein the fluid sample is selected from the group consisting of a breath sample, a saliva sample, a perspiration vapor sample and a gastric reflux sample.

6. The method of claim 1 wherein the fluid sample is a breath sample.

7. The method of claim 6 wherein the breath sample is an exhaled sample or multiple breath samples.

8. The method of claim 1 wherein the aqueous solution further comprises sodium hydroxide.

9. The method of claim 1 wherein a droplet of water placed on the surface of the hydrophobic polymer has a contact angle of not less than about 100 degrees.

10. The method of claim 1 wherein the polymer has an average pore size of less than about 9 microns.

11. The method of claim 10 wherein the polymer has an average pore size of about 1.0 microns to about 2.5 microns.

12. The method of claim 1 wherein the polymer is selected from the group consisting of polytetrafluoroethylene, polyvinylidene difluoride, acrylic-based polymers, ethylene propylene polymers, polycarbonate, polyproylene, polyvanilidine chloride, dimethyl polysiloxane.

13. The method of claim 1 wherein the polymer comprises polyvinylidene difluoride.

14. The method of claim 1 wherein the dye is selected from the group consisting of bromophenol blue, bromothy-

mol, phenol red, methyl orange, methyl yellow, 2,4-dinitrophenol, 2,6-dinitrophenol, and cresol red.

15. The method of claim 1 wherein the dye is bromophenol blue.

16. The method of claim 1 wherein the sensor changes color within about 5 to about 60 seconds after the porous polymer is permeated with the ammonia gas.

17. The method of claim 1 wherein the subject is administered a mouth wash comprising an antibacterial agent prior to collection of the breath sample to reduce the number of bacteria present in the subject's mouth.

18. The method of claim 1 wherein the concentration of ammonia gas is determined by measuring the color change of the dye with an optical reader.

19. The method of claim 1 wherein the sensor is in the form of a membrane.

20. The method of claim 1 wherein the sensor is in the form of particulate matter.

21. A method to detect the presence of a bacteria in the gastrointestinal tract of a subject, which when present in the gastrointestinal tract of the subject is associated with the catalytic degradation of urea to ammonia and carbon dioxide, the method comprising:

(a) delivering a source of urea to the gastrointestinal tract of the subject,

(b) obtaining a liquid sample from the subject after the delivery of the urea source,

(c) contacting the liquid sample or a derivative thereof with a sensor, the sensor comprising a hydrophobic polymeric material and a dye associated with the polymeric material, the dye having the capacity to become deprotonated and undergo a color change in the presence of ammonia,

(d) controlling the contact conditions such that the sensor responds to the presence of ammonia in the fluid sample but not to the pH of the fluid sample by undergoing an optically discernible color change, and

(e) optically detecting a color change in the sensor after the sensor is contacted with the fluid sample.

22. The method of claim 21 wherein the liquid sample is selected from the group consisting of a saliva sample and a tear sample.

23. A method to detect the presence of a bacteria in the gastrointestinal tract of a subject, which when present in the gastrointestinal tract of the subject is associated with the catalytic degradation of urea to ammonia and carbon dioxide, the method comprising:

(a) delivering a source of urea to the gastrointestinal tract of the subject,

(b) inducing the subject to exhale a breath sample into a container, the container comprising an inlet, a vent, and a sensor, and being adapted to restrict the flow of gas out of the inlet, the sensor comprising a hydrophobic polymeric material and a dye associated with the polymeric material, the dye having the capacity to become deprotonated and undergo a color change in the presence of ammonia,

(d) controlling the contact conditions such that the sensor responds to the presence of ammonia in the breath sample but not to the pH of the breath or any liquid in the container by undergoing an optically discernible color change, and

(e) optically detecting a color change in the sensor after the subject exhales into the container.

24. The method of claim 23 wherein a droplet of water placed on the surface of the hydrophobic polymer has a contact angle of not less than about 100 degrees.

25. The method of claim 23 wherein the polymer has an average pore size of less than about 9 microns.

26. The method of claim 23 wherein the polymer has an average pore size of about 1.0 microns to about 2.5 microns.

27. The method of claim 23 wherein the polymer is selected from the group consisting of polytetrafluoroethylene, polyvinylidene difluoride, acrylic-based polymers, ethylene propylene polymers, polycarbonate, polyproylene, polyvanilidine chloride, dimethyl polysiloxane.

28. The method of claim 23 wherein the polymer comprises polyvinylidene difluoride.

29. The method of claim 23 wherein the dye is selected from the group consisting of bromophenol blue, bromothymol, phenol red, methyl orange, methyl yellow, 2,4-dinitrophenol, 2,6-dinitrophenol, and cresol red.

30. The method of claim 23 wherein the dye is bromophenol blue.

31. A breath sampler for use in detecting the presence of *H. pylori* in the gastrointestinal tract, the breath sampler comprising a breath handler and a detection unit including a sensor capable of reacting to the presence of an indicator in the breath sample of *H. pylori* in the gastrointestinal tract, the breath handler being adapted to route air through the breath sampler upon inhalation by the subject to the subject while substantially blocking flow of air through the detection unit to the subject, and being adapted upon exhalation of the subject to route exhaled air through the breath handler to the detection unit for detection of *H. pylori* in the gastrointestinal tract.

32. A breath sampler as set forth in claim 31 wherein air flow through the detection unit is substantially blocked except upon exhalation by the subject through the breath sampler.

33. A breath sampler as set forth in claim 32 wherein the detection unit is substantially isolated from ambient except upon exhalation by the subject through the breath sampler.

34. A breath sampler as set forth in claim 32 wherein the breath handler includes an air intake, a breath sample opening and a breath sample outlet, the detection unit being arranged to receive air from the breath handler by way of the breath sample outlet, the breath handler receiving ambient air through the air intake upon inhalation by a subject and directing air to the breath sample opening for inhalation by the subject, and blocking a path from the breath sample outlet to the breath sample opening, the breath handler directing exhaled air from the subject to the breath sample outlet and thence to the detection unit, and blocking a path from the breath sample opening to the air intake.

35. A breath sampler as set forth in claim 34 wherein the breath handler comprises a tube having the air intake at one end thereof and the breath sample opening at an opposite end thereof, and a collection branch in fluid communication with the tube and the detection unit and including the breath sample outlet.

36. A breath sampler as set forth in claim 35 wherein the breath handler further comprises a first valve located gen-

erally at the air intake end of the tube for permitting inhaled air to flow into the tube but blocking the breath sample from flowing out of the tube through the air intake, and a second valve located in the collection branch for permitting the breath sample to flow from the tube through the collection branch and into the detection unit and blocking flow of air from the detection unit through the collection branch into the tube.

37. A breath sampler as set forth in claim 36 wherein the detection unit includes a vent for exhausting excess exhaled air from the detection unit, and wherein the detection unit further comprises a third valve disposed for permitting exhaust of exhaled air under pressure through the vent from the detection unit and inhibiting the intake of ambient air through the vent from outside the detection unit.

38. A breath sampler as set forth in claim 37 wherein the first, second and third valves are one way valves biased to a closed position in the absence of an air pressure differential across the valves.

39. A breath sampler as set forth in claim 31 further comprising liquid in the detection unit, the breath handler being adapted to discharge the breath sample into the liquid.

40. A breath sampler as set forth in claim 31 wherein the detection unit includes a vent for exhausting excess gas from the detection unit, and the breath handler comprises a tube having an intake end including an air intake and a mouthpiece portion including a breath sample opening, the intake end being positioned to locate the air intake out of registration with the vent to inhibit inhalation of exhausted air from the detection unit.

41. A breath sampler as set forth in claim 40 wherein the breath handler is pivotally mounted in the detection unit.

42. A breath sampler as set forth in claim 31 wherein the breath handler comprises a tube having an intake end including an air intake and a mouthpiece portion including a breath sample opening, the mouthpiece portion being tapered toward the breath sample opening to facilitate reception into the back of the mouth near the throat.

43. A breath sampler as set forth in claim 31 wherein the detection unit comprises a collection receptacle and the sensor is mounted in the collection receptacle, the sensor being adapted to change color upon the detection of said indicator in the breath sample, and the collection receptacle being at least partially transparent to permit a line of sight to the sensor.

44. A breath sampler as set forth in claim 31 wherein the breath sample outlet is arranged relative to the detection unit for directing the breath sample against the sensor.

45. A breath sampler as set forth in claim 31 in combination with a reader arranged for reading the sensor of the detection unit and displaying a readout corresponding to the sensor indication, the reader having a self contained power source and being adapted for connection together with the breath handler and detection unit when used by the subject to obtain a breath sample.

46. The combination as set forth in claim 45 wherein the reader is formed with a handle for holding the reader, detection unit and breath handler as the breath sample is being obtained.

47. The combination as set forth in claim 46 further in combination with a supply of urea in the form of at least one of: a bottle of urea and at least one tablet of urea.

48. A breath sampler for use in detecting the presence of *H. pylori* in the gastrointestinal tract, the breath sampler comprising a breath handler for receiving a breath sample from a subject, and a detection unit operatively connected to the breath handler for receiving the breath sample from the breath handler, the detection unit comprising a collection receptacle, a sensor membrane disposed in the receptacle capable of reacting to the presence of ammonia in the breath sample indicating the presence of *H. pylori* in the gastrointestinal tract, and a liquid in the collection receptacle generally covering the membrane, the liquid being capable of capturing ammonia from the breath sample to retain it in proximity to the sensor membrane.

49. A breath sampler as set forth in claim 48 wherein the liquid is water.

50. A breath sampler as set forth in claim 48 wherein the collection receptacle includes a bottom wall, the sensor membrane being disposed on the bottom wall and covered by the liquid.

51. A breath sampler for use in detecting the presence of *H. pylori* in the gastrointestinal tract, the breath sampler comprising a breath handler for receiving a breath sample from a subject, the breath handler including a mouthpiece portion having a breath sample opening in a distal end thereof, the mouthpiece portion being shaped for reception in a subject's mouth to locate the breath sample opening proximate the throat, and a detection unit operatively connected to the breath handler for receiving the breath sample from the breath handler, the detection unit including a sensor for detecting an indicator present in the breath sample of *H. pylori* in the gastrointestinal tract.

52. A breath sampler as set forth in claim 51 wherein the mouthpiece portion tapers in at least one dimension toward the breath sample opening.

53. A breath sampler as set forth in claim 52 wherein the mouthpiece portion tapers in cross sectional width and height.

54. A breath sampler as set forth in claim 53 wherein the breath handler comprises a tube including the mouthpiece portion and breath sample opening, and a collection branch extending from the tube to the detection unit, the mouthpiece portion extending from the collection branch to the breath sample opening and having a length selected to extend to the back of the subject's mouth.

* * * * *

L11: Entry 2 of 9 File: PGPB Nov 13, 2003

DOCUMENT-IDENTIFIER: US 20030211618 A1
TITLE: Color changing steam sterilization indicator

CLAIMS:

8. The device of claim 7 wherein said indicator comprises at least one member chosen from the group consisting of acid alizarin violet N, acid blue 113, acid blue 93, acid red 88, auramine-O, azures, benzo purpurin 4B, bismarck brown Y, brilliant crocein MOO, bromocresol purple, bromophenol blue, bromopyrogallol red, chrysoidin, chrysophenine, Congo red, darrow red, dimethylmethylene blue, direct blue 71, direct red 75, disperse blue 14, eriochrome blue black B, Guinea green B, indoline blue, Janus green B, leuco crystal violet, meta-cresol purple, methylene blue, methythymol blue, naphthochrome green, Nile blue chloride, oil blue N, pararosaniline acetate, pyrogallol red, quinoline yellow, rhodamine, solvent blue 59, solvent green 3, and thionin.

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(19) United States

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(54) COLOR CHANGING STEAM STERILIZATION INDICATOR

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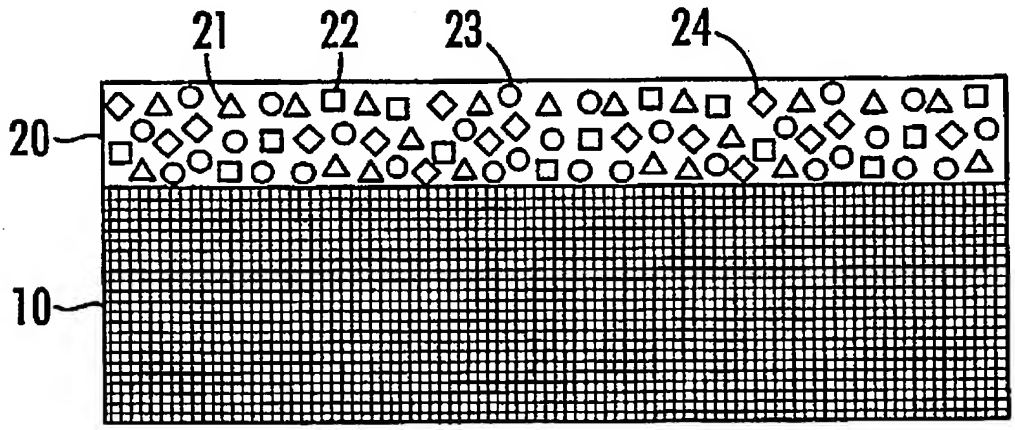
(52) U.S. Cl. 436/38; 422/56

(57) ABSTRACT

There is provided a device (10) for monitoring sterilization of a material with steam comprising at least one layer (20), having incorporated therein an isomeric indicator (22), capable of undergoing at least one color change and optionally a controller (23) for said indicator capable of influencing the time and temperature required for said color change when contact with steam. Composed of polymeric binder (21).



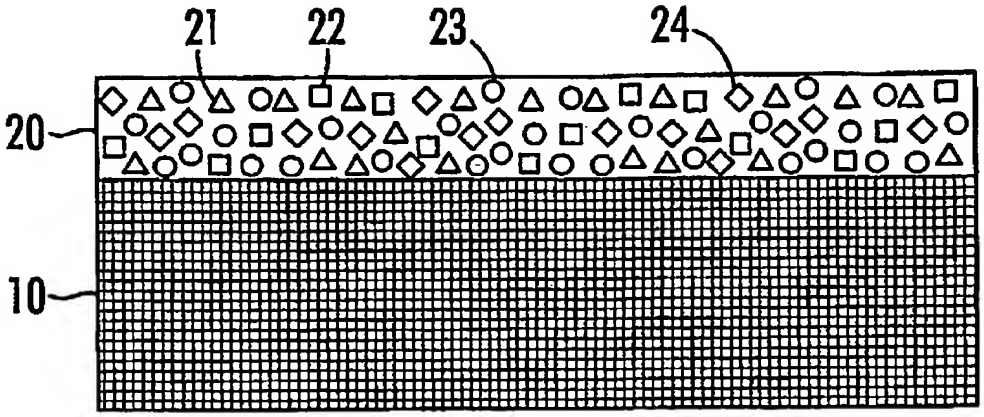


FIG. 1

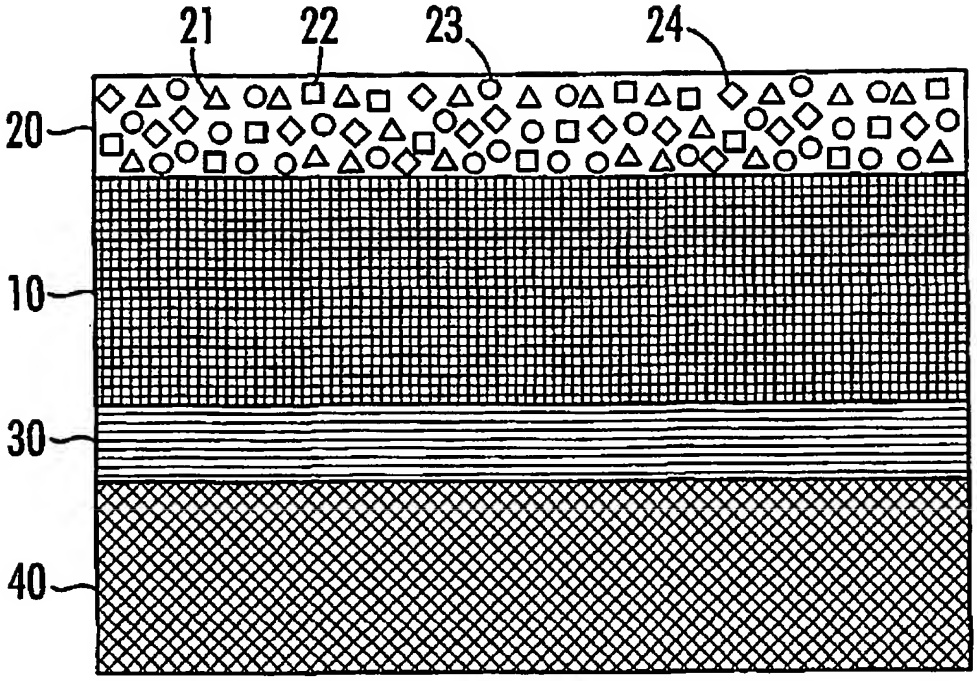


FIG.2

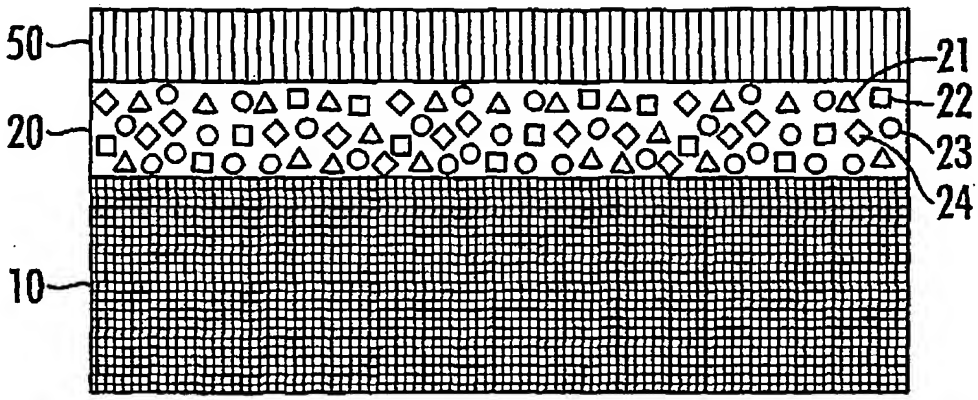


FIG.3

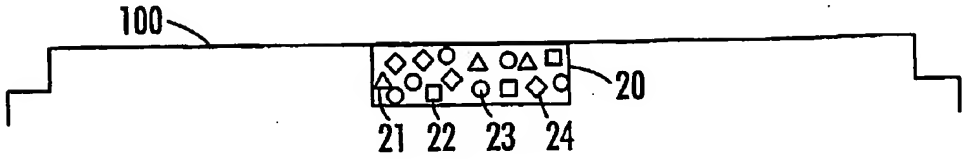


FIG. 4

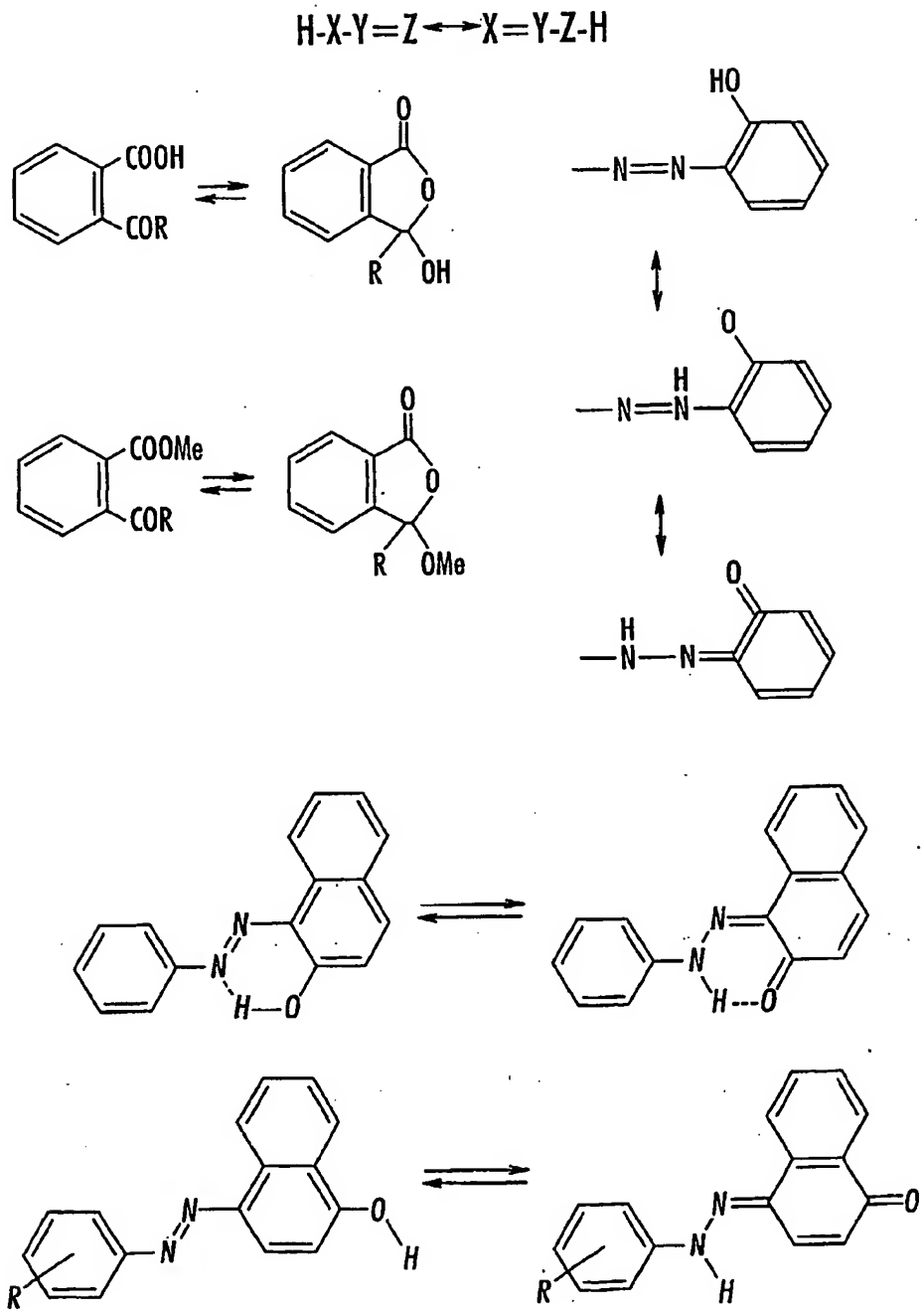
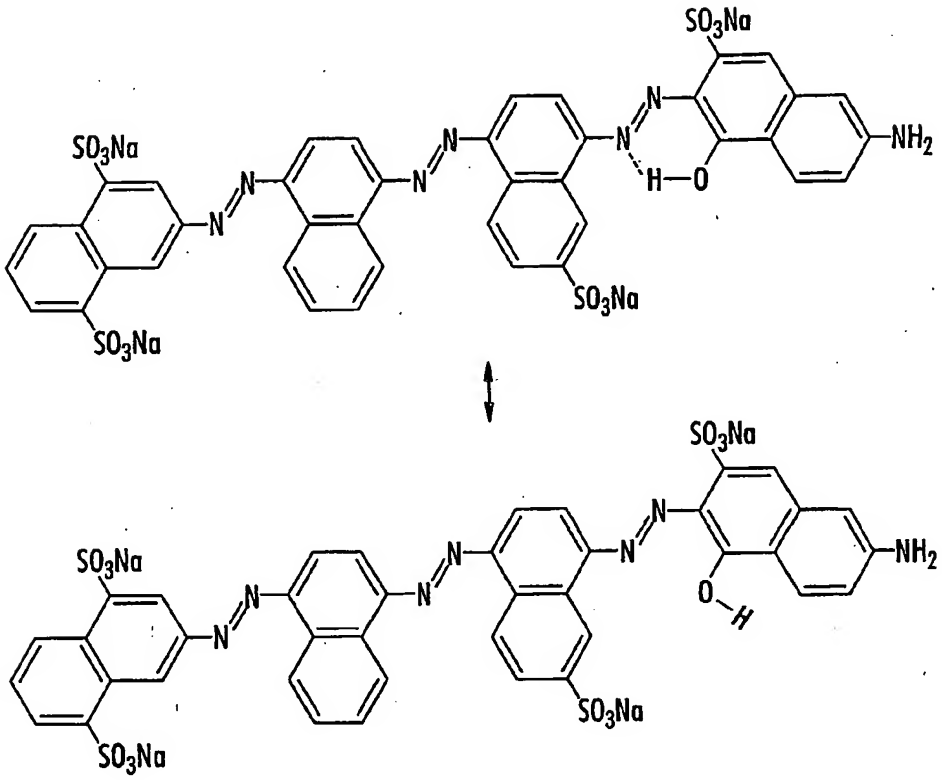


FIG. 5


SO3Na
SO3Na
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NH2
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H
NH2

FIG.6

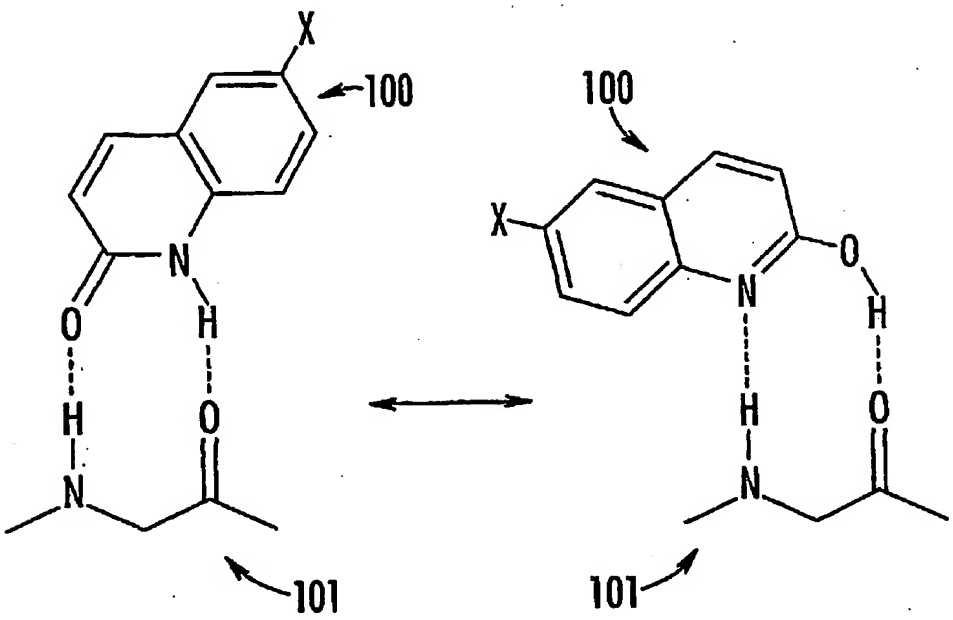

FIG. 7

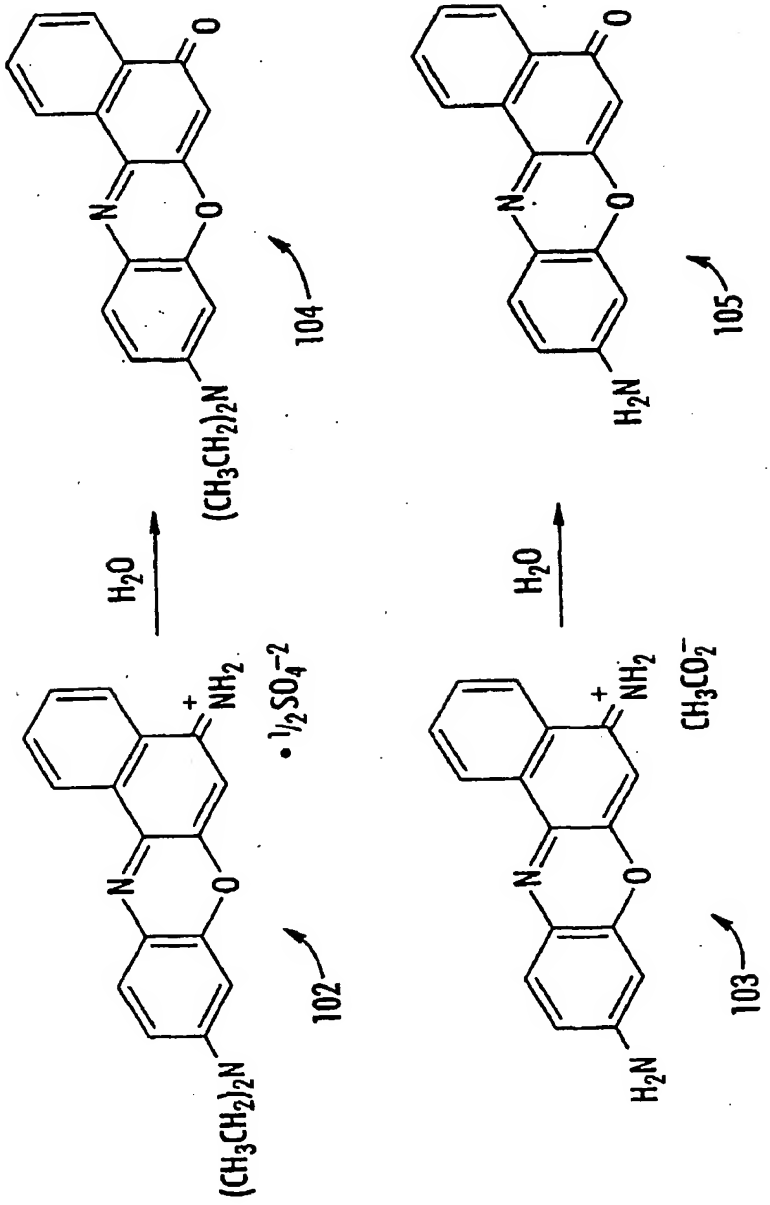
O
N
O
$(CH_3CH_2)_2N$
104
H_2O
NH_2
$\cdot \frac{1}{2} SO_4^{-2}$
102
H_2N
105
$CH_3CO_2^-$
103

FIG. 8

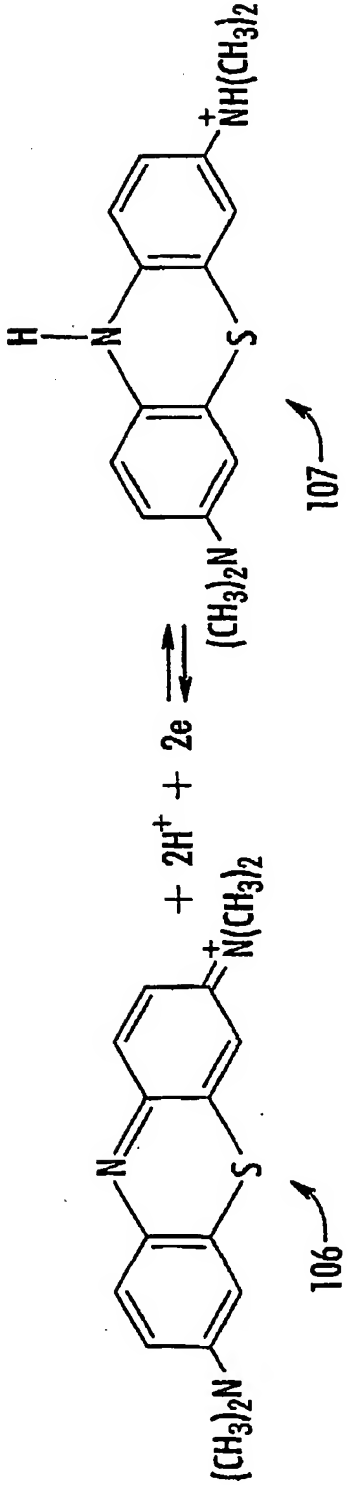

FIG. 9

COLOR CHANGING STEAM STERILIZATION INDICATOR

BACKGROUND OF TIE INVENTION

[0001] 1. Field of the Invention

[0002] The present invention relates to a color-changing device for monitoring integral value of time, temperature and steam. The device can be used for monitoring sterilization of medical and kitchen supplies, canned foods and doneness of microwave foods.

[0003] 2. Brief Description of Prior Art

[0004] A wide variety of medical supplies are sterilized with materials and techniques, such as steam, ethylene oxide, plasma, peracetic acid, formaldehyde and high-energy radiation. Kitchenware, such as dishes, cutlery, and utensils used at home and restaurants are also sterilized in dishwashers with hot water and hot air usually around 90° C. It is essential to assure that these items are sterilized. A number of indicators, dosimeters and monitors are proposed in the literature. They include biological and chemical indicators. The color changing chemical indicators are inexpensive and are widely used.

[0005] In order to assure the sterilization with steam, the indicator, or dosimeter, must determine integral value of three parameters viz. time, temperature and steam. It is often desirable that the indicator is essentially unaffected by other parameters, such as dry heat, humidity, steam, ethylene oxide and radiation.

[0006] Pre-cooked frozen food is widely used today. The pre-cooked frozen food is heated either in a conventional oven (for example, heated with natural gas or electricity) or more conveniently in a microwave oven. A microwave oven does not heat the food uniformly. Some portions of food may not be done while the other portions may be over heated. Hence, there is a need for an indicator that changes color when steam is emitted by the food.

[0007] Homes, restaurants and catering organizations use kitchenware such as dishes, cutlery and utensils, which need to be sterilized with either dry heat, hot water and steam usually below 100° C. There is also a need for an indicator to make sure that the cookware has been subjected to certain integral value of heat and/or humidity.

[0008] A wide variety of foods especially canned foods, pharmaceuticals, hospital and medical supplies are sterilized. These and other products such as linens are sterilized to kill living organisms to an acceptable level. Direct testing for sterility is destructive and expensive and hence indirect testing methods, such as color changing indicators are used.

[0009] Biological indicators made from cultures, such as Bacillus subtilis spores, bacillus pumilus spores and clostridiumn sporogenes spores are used for monitoring the sterilization. However, chemical indicators are widely used because they are simple and inexpensive.

[0010] Many steam sterilization indicators are reported in the literature and some of them are used for monitoring sterilization. A few of them use heavy and toxic metals, such as lead. There is a need for a sterilization indicator that does not use toxic and heavy metals.

[0011] U.S. Pat. No. 3,523,011 describes an indicator material consisting of calcium sulfide and lead carbonate. Upon exposure to steam at ~120° C., calcium sulfide decomposes to form calcium hydroxide and hydrogen sulfide. The hydrogen sulfide reacts with lead carbonate to form black lead sulfide. Steam sensitive composition of U.S. Pat. No. 5,064,576 contains a metal complex (e.g. zirconium chloranilate) and an exchange ligand (e.g. citric or tartric acid salts and amino carboxylic acid), binder (e.g. nitrocellulose and ethylcellulose) and a color change rate regulator (e.g. Resino blue, Resino yellow). U.S. Pat. No. 4,514,361 discloses a steam sterilization indicator containing a carrier (e.g. filter paper), a pH value indicator (e.g. bromocresol purple) and a chemical composition that contains (a) 2,4-dihydroxybenzoic acid and its metal salt and (b) phenylpropionic acid and its metal salt. Under steam sterilization conditions, the pH of mixture exceeds pre-determined pH (5.8 to 6.2) due to formation of carbonate or bicarbonate (basic), causing the indicator to change color and indicate that sterilization is complete. U.S. Pat. No. 5,158,363 describes a steam sterilization indicator, which contain (a) water-soluble organic compound whose melting point in the absence of steam is greater than sterilization temperature and (b) ink dye. Upon steam exposure, dye changes color from clear to dark brown or black. U.S. Pat. No. 5,087,659 describes ink composition as steam sterilization indicators for use in jet printing. The composition uses an organic dyestuff, which forms a salt with phenol resin. The ink composition is discolored or changes color under steam sterilization conditions. U.S. Pat. Nos. 3,981,683, 3,932,134, 4,195,055 and 4,410,493 illustrate processes, which use permeation or wicking of an indicator chemical (such as sebasic acid and salicylamuide) and a dye. A disposable pre-vacuum steam sterilizer test device is described in U.S. Pat. No. 4,486,387. Other indicators for noting the completion of steam sterilization are reported in U.S. Pat. Nos. 4,121,714; 3,360,339; 2,826,073; 3,568,627; 3,360,338; 2,798,885; 3,386,807; 3,360,337; and 3,862,824. The indicators, which monitor integral value of time, temperature and humidity are often commonly referred to as steam indicators herein.

[0012] Patel in PCT application number # WO 01/10471 A1 has disclosed ink formulations and devices for monitoring sterilization with ethylene oxide. The device is made by coating a mixture of (a) a polymeric binder, (b) a ethylene oxide reactive salt, such as sodium thiocyanate and tetraethylammonium bromide and (c) a pH sensitive dye, such as bromothymol blue and bromocresol purple. When contacted with ethylene oxide, the device undergoes at least one color change due to production of a base such as sodium hydroxide. However, these devices and formulations are selective to ethylene oxide only.

[0013] Patel in PCT application # WO 00/61200 has disclosed formulations and devices for monitoring sterilization with plasma. The device is made by coating of a mixture of at least one (a) polymeric binder, (b) plasma activator and (c) plasma indicator. The device undergoes a color change when treated with plasma, especially that of hydrogen peroxide. For example, when a coating of phenol red and tetraethylammonium bromide in a binder, such as polyacrylate undergoes a color change from yellow-to-blue when exposed to hydrogen peroxide and its plasma due to halogenation of the dye. However, these devices and formulations are selective to plasma only.

[0014] Even though equilibrium processes which include reversible reactions, interconversions such as migration of atoms such as hydrogen, structural changes, isomerizations, configuration changes such as cis ⇆ trans, stereoisomerisation, isomeric transitions, polymorphism, isomorphism, phase changes and tautomerism are known, there is no report on use of such processes and compounds undergoing isomerization for monitoring integral value of time, temperature and moisture.

[0015] Many dyes and pigments gets oxidized and reduced with oxidizing and reducing agents respectively. Oxidation and reduction processes are often associated with a color change. These dyes are usually known as redox dyes. Some examples of redox dyes are: neutral red, dimethylindoaniline, indigodisulfonic acid, nile blue A, methylene blue, thionin, brilliant cresyl blue, dichloroindophenol, dimethoxybenzidine, diphenylbezidine, diphenylamine, o-toludine, bezopurprin 4B and naphthol blue black. A number of color changing redox systems are summarized in a book by E. Bishop [for example, see chapter 8 in "Indicators", E. Bishop (Ed), Pergamon Press, Oxford, 1972]. Chapter 7 of this book describes some adsorption indicators. Adsorption indicators which change color with humidity can be used as steam indicators.

[0016] A number of dyes also change color with solvents, usually with change in polarity of the solvent, hydrogen bonding, donation and acceptance of electron pairs Some dyes also change color when dissolved. These dyes are commonly referred to as solvatochromic dyes. Solvatochromic dyes are summarized in a review by C. Reichardt [Chemical Reviews, 94, 2319-2358 (1994) and references quoted therein]. An example of solvatochromic dyes is N-phenoxide betaine, Michler's ketone, Nile red, phenol blue, iron phenanthroline and some mecrocyanine, and stilbenzonium dyes.

[0017] The above referred processes are collectively or individually referred herein to as isomerization process(es) and compound(s) undergoing isomerization as isomer(s). The isomers, including dyes which change colors, when they transform from one isomeric form to the other, without going through a major chemical reactions, are collectively and/or individually referred herein as to isomeric indicators or simply indicators.

SUMMARY OF THE INVENTION

[0018] It is an object of the present invention to provide an indicator which can monitor integral value of temperature, time and water vapor. It is another object of the present invention to provide an indicator which is economical to manufacture and use.

[0019] Provided is a device made by coating a mixture of (a) a polymeric binder, (b) an isomeric indicator and optionally (c) a controller which controls the time and temperature of isomerization of the said indicator, when contacted with water vapor, undergoes at least one color change. Such a device can be used for monitoring sterilization of medical supplies and canned foods, and doneness of microwave foods.

[0020] Also provided is a device for monitoring integral value of time, temperature and water vapor comprising at least one layer of polymer, having incorporated therein (a) an isomeric indicator capable of undergoing at least one color change and optionally (b) a controller for said indicator wherein said indicator, when contacted with water vapor, undergoes an isomerization reaction which causes said indicator to undergo said color change.

[0021] The indicators suitable for use in this device include pigments, dyes, precursors of them, and their mixtures. A desirable quality of the indicator is the ability to undergo a color change upon isomerization, with or without a controller, when contacted with water vapor. Desirably the indicator undergoes a yellow-to-blue, yellow-to-green, red-to-yellow, red-to-green, red-to-blue or vice versa color change. Suitable indicators include dyes having ability to isomerize, or change to a tautomer or formation or breaking a hydrogen bond, get oxidized or reduced, or get dissolved.

[0022] A preferred polymer used in the device is, suitably, soluble in water or dispersible in an aqueous medium solvent. The polymer can also be formed by polymerization with high energy radiation, such as UV and electron beam. A broad class of polymers may be used. They may be homopolymers, copolymers or a mixture thereof, suitably a vinyl or olefin polymers, such as that of styrenes, acrylates, acrylic acid, acrylamide, vinyl acetate, vinyl alcohol, vinyl chloride, epoxide, polyurethanes, cellulose nitrate, carboxyethyl cellulose or a mixture thereof. Desirably, the polymer is an acrylate polymer, polyurethane, cellulose nitrate or carboxymethylcellulose.

[0023] Preferably the controller is a compound having the ability to influence the rate and temperature of isomerization of said indicator when contacted with water vapor. The nature of controller depends upon the isomerization process. Controller could also be an isomeric compound e.g., a derivative or complex of acetoacetic acid and 2,4-pentanedione (acetylacetone). Suitable controllers are tautomers or compounds having ability to form hydrogen bonds, dissolve, oxidize or reduce the indicator. Desirable controller includes acids, acetylacetonates, aldehydes, alcohols, amides, amidrazones, amines, azo, dithiocarbamates, esters, hydrazides, hydrazones, hydroxyamines, imidazoles, imadozolines, imides, imines, indolines, ketones, lactams, lactones, nitramides, nitriles, nitrones, oximes, pentanediones, phosphates, phthalides, pyrimidines, semicarbazones, thiophenes, thioureas, triazenes, triazoles, ureas, solid solvents, oxidizing and reducing agents or a mixture thereof. Controller may not interact with said indicator or may stabilize the isomeric form under normal ambient conditions. Other additives may be used to stabilize the ambient form or the steam treated form of said indicators.

[0024] The process of making a device of the present invention comprises dissolving or dispersing the components such as the indicator, controller and binder in a solvent thereof, applying the thus formed solution/dispersion to a substrate and permitting the solvent to evaporate. The process also includes dissolving or dispersing the components in monomers and oligomers polymerizable with high energy radiation, such as UV light and electron beam, and curing them to a polymer with such radiation.

[0025] The substrate may be a container for an item to be sterilized. It may also be a plastic film, paper or metal, including but not limited to polyester film, paper or spun bonded polyolefins.

[0026] In a desirable embodiment of the invention is a solution or dispersion of an ink formulation suitably an aqueous ink formulation most suitably one, which comprises an acrylate polymer.

[0027] A process of using a device of the present invention for monitoring sterilization of materials and doneness of food comprises the steps of affixing the device to said materials or containers containing same, carrying out the process of sterilization including the step of exposing the device to water vapor including high pressure steam and observing the presence of a color change of said device.

[0028] A particularly preferred embodiment is provided in a device for monitoring integral value of time, temperature and water vapor. The device comprises at least one layer of polymer comprising an indicator wherein an isomeric indicator capable of undergoing at least one color change and 0-50%, by weight, a controller for the indicator which is capable of influencing the time and temperature required for the color change to occur when contacted with water vapor. The indicator undergoes an isomerization reaction which causes the indicator to undergo said color change.

[0029] Yet another embodiment is provided in a process of making a device comprising:

[0030] a) dissolving or dispersing an indicator in a solvent to form a solution;

[0031] b) applying the solution to a substrate; and

[0032] c) permitting the solvent to evaporate.

[0033] Also provided is ink formulation for making the devices for monitoring integral value of time, temperature and water vapor, comprising polymeric binder, solvent, indicator and controller.

BRIEF DESCRIPTION OF THE DRAWINGS

[0034] **FIG. 1.** A side schematic cross section of one embodiment of the steam sterilization indicator of the invention wherein an indicator layer comprised of a polymeric binder, isomeric indicator and optionally a controller for the indicator is applied on a substrate.

[0035] **FIG. 2.** A side schematic cross section of the steam sterilization indicator of the invention having an adhesive layer and a release layer.

[0036] **FIG. 3.** A side cross-section of a multi-layer device wherein a top layer is a coating or lamination as a barrier.

[0037] **FIG. 4.** A side schematic cross section of another embodiment of the steam indicator device which is substantially the same as that in **FIG. 1** except that the device is applied under the lid of a microwave food container.

[0038] **FIG. 5.** An equation for a tautomeric reaction and examples of azo ⇆ hydrazo tautomers.

[0039] **FIG. 6.** Chemical structures of two isomers of direct blue 71.

[0040] **FIG. 7.** Formation of two isomers by interaction with a controller.

[0041] **FIG. 8.** Metachromism of Nile blue A and Cresyl violet acetate introduced by water.

[0042] **FIG. 9.** Oxidized and reduced form of methylene blue.

BRIEF DESCRIPTION OF THE INVENTION AND PREFERRED EMBODIMENTS

[0043] The device can be best described by reference to the Figures. As shown in **FIG. 1**, the device in one of the simplest form is comprised of an indicator layer X, applied on a substrate **10**. The substrate **10** can also be a container, such as pouch or can for products to be sterilized or a food container. The indicator layer **20** is composed of a polymeric binder **21**, and containing at least one isomeric indicator **22**, capable of undergoing a color change when contacted with steam. The indicator layer **20** may optionally contain a controller **23** to control e.g., the time and temperature required for the color change. The indicator layer **20** may contain other additives **24** such as a stabilizer for the isomeric indicator, crosslinking agent or UV absorber.

[0044] As shown in **FIG. 2**, the substrate **10** of the device can optionally be a film or paper coated with an adhesive layer **30**. The adhesive layer allows the device to be affixed to a container of product to be sterilized. To the bottom of the adhesive layer **30**, can be affixed a release layer **40** for ease in packaging and for removal just prior to use. Removal of the release layer **40** will enable the entire device to be affixed to the container of product to be sterilized.

[0045] The device can be composed of more than one layer. The device could have two indicator layers. As shown in **FIG. 3**, in its simplest form of the multi-layer device, the second top layer **50** could be a barrier for steam, e.g., a polymeric coat or a laminated film, on to layer **20**. The barrier layer **50** can reduce diffusion of steam, thereby increasing the time required for the color change. The top layer **50** can be a transparent laminated film. The indicator layer can also be sandwiched between two films or between a paper and a film.

[0046] A moving boundary device can be created if the barrier layer **50** is in the form of a wedge over the indicator layer **20**. The barrier layer will resist but will be permeable to steam.

[0047] The device can be used for monitoring doneness of microwave and other foods. The device of **FIG. 1** or **FIG. 2** can be applied under a lid of the microwave food container. **FIG. 4** is a side schematic cross section of another embodiment of the device where the indicator layer **20** is applied under the lid **100** of a microwave food container.

[0048] Other variations of the steam indicator device are also possible, for example, a gradient device can be created by coating a series of formulations having the time required for the color change either increases or decreases. Such gradient can be obtained by coating such formulations in form of lines or bars next to each other.

[0049] The device could also be created by printing the indicating formulation in form of a number, image, bar code or message, e.g., "if this print is green, the product inside is sterilized".

[0050] An example of a keto-enol, amido-imido, thiol-thione, type isomerization reaction (H—X—Y=Z⇆ X=Y—Z—H) of the present invention is shown in **FIG. 5**. A typical example is acetoacetic acid and its derivatives

which can exist as a keto ($CH_3COCH_2COOCH_2CH_3$) and enol ($CH_3C(OH)=CHCOOCH_2CH_3$) forms.

[0051] A typical example of dye, which can have two tautomers, is direct blue 71 as shown in **FIG. 6**. The tautomer which provides blue may be hydrogen bonded (between —O—H and —N=N—) and the hydrogen bonds may be broken in the other tautomer, which would provide red or purple color. The delocalized electrons of the dye molecule can travel over a longer (longer effective conjugation) length in the hydrogen bonded form and hence would appear blue. Once the hydrogen bond is broken, the segments of the molecule can rotate along the single bonds and the molecule may become slightly nonplannar. Such nonplannar molecule would have shorter effective conjugation length and would appear red or purple color.

[0052] The isomerization of the indicator can also be introduced by interaction with another additive or controller. **FIG. 7** shows an isomerization of an indicator molecule, **100**, with the amide functionality interacting with a controller molecule, **101**, by hydrogen bonding. A color change may be noticeable under UV light.

[0053] **FIG. 8** illustrates isomerization (metachromism) of Nile blue A, 102, to Nile red, 104, and Cresyl violet acetate, 103, to crystal red, 105, which can be introduced by water, water vapor and steam.

[0054] Oxidized (blue colored) and reduced form (colorless) of methylene blue are shown in **FIG. 9**. A large number of dyes exhibit oxidized and reduced forms. Oxidation can be introduced with an oxidizing agent as a controller and reduction can be introduced with reducing agent as a controller.

[0055] The feasibility of the concept was demonstrated by using an acrylate printing ink extender 001270 supplied by Environmental Inks and Coating, Co, Lithicum, Md. as a polymeric binder, direct blue 71 as an indicator and tetramethylhexane diamine as a controller for the indicator. The acrylate ink extender 001270 is referred herein to as EC001270. The mixture of direct blue 71 and tetramethylhexane diamine is purple color in EC001270 and changes to blue when treated with steam. Compounds such as tetramethylhexane diamine, which form or stabilize one of the isomer (e.g., the red/purple color of direct blue 71) are also referred to herein as controllers.

[0056] Tautomerism by the way of example of classes also includes acylotropic, alkylotropic, cabonotropic, phosphoryltropic, silylotropic, vinyltropic, and valance tautomerism including π, σ,π-valence tautomerisms. Polar multiple bonds such as C=O, C=N, N=C, N=N, C=C, C≡N, and C≡C are capable of intramolecular addition of many functional groups such as O—H, N—H, S—H, CO-halogen. Typical examples of such intramolecular reaction include keto-enol, amido-imidol, thiol-thione, and benzoid-quinone. Examples of classes of compounds suitable for indicator and controller include acids, aldehydes, alcohols, amides, amidrazones, amines, azo, dithiocarbamates, esters, hydrazides, hydrazones, hydroxyamines, imidazoles, imadozolines, imides, imines, indolines, ketones, lactams, lactones, mercapto, nitramides, nitriles, nitrones, oximes, pentanediones, phosphates, phthalides, pyrimidines, semicarbazones, thiophenes, thioureas, triazenes, triazoles, and related compounds and derivatives or mixture thereof. Compounds having these functionalities can be used as controllers.

[0057] With reduced dyes, oxidizing agents can be used as controllers. Oxidants such as nitrates, nitrites, peroxides, dimethylsulfide, dimethylsulfoxide, hydrogen peroxide-urea complex, carbon tetrachloride, peroxyacids, amine-oxides, alkyl nitrates, alkyl nitrite, complexes of halides such as bromine, per-iodates, per-haloacids and haloates, e.g., perchloric acid and sodium perchlorate, persulfates, e.g., sodium persulfate, metals and metal oxides can be used as oxidants for the device. Oxidants are described in "Oxidation in Organic Chemistry" M. Hudlicky, ACS Washington D.C., Monogram #186, 1990.

[0058] With the oxidized form of dyes, reducing agents can be used as controllers. Several classes of reducing agents such as hydrazines, nitrites, thiocyanates, sulfite, sulfides, reduced metal salts, oximes and unsaturated compounds are suitable as reducing agents. Examples of reducing agents are: ammonium sulfite, ammonium thiocyanate, calcium ferrocyanide, Fe(II) salts, sodium bisulfite, sodium cyanate, sodium dithionite, sodium hydrosulfide, sodium sulfite, sodium thiocyanate, sodium thiosulfate, acetone Oxime, benzoquinone dioxime, cupferron, cyclopenatanone oxime, diphenylglyoxime, salicyladoxime, and ascorbic acid.

[0059] Solvents for the solvatochromic dyes could be solids or high boiling liquids. Solid solvents are preferred. A powder, e.g, in the form of fine particles, of a solid compound can be mixed with a solvatochromic dye in binder. Upon heating the compound can melt and/or get dissolved and then can dissolve the dye. The dissolution of dye/indicator may be associated with a color change. A large number of compounds, especially organic compounds, which are solid under ambient conditions and get melted or dissolved with water vapor at a higher temperature can be used as controllers for the solvatochromic indicators. The examples of such compounds includes, phenols, polyalcohols, acids, amines, esters, amides, e.g., benzoic acid, diphenyl butyro lactone, glucose pentaacetate, glyconolactone, inositol, chlorinated paraffins, trichlorobenzylacetate, trichloroacetamide, vitamin-c palmitate, tribenzylamine, salicylanamid, hexachloro norborene dicarboxylic acid, and methyldinitrosalicylate.

[0060] Any material, which undergoes a color change when treated with water, water vapor including high-pressure steam due to isomerization, with or without indicator controller, can be used as a steam indicator. Steam indicators are also referred herein to as indicators. Most preferred classes of steam indicators are dyes, pigments and their precursors. The dyes having more than one isomers are preferred. Still preferred are the dyes having ability to form and break hydrogen bond or migration of hydrogen atom (process generally preferred as tautomerization). Another class of preferred indicators are dyes which can be oxidized and reduced. Still another class of preferred class of indicators are solvatochromic dyes that can change color when dissolved.

[0061] A wide variety of dyes such as nitroso, nitro, azo (mono, di, tri and polyazo), azoic, stilbene, carotenoid, diphenylmethane, triphenylmethane, xanthene, acridine, quinoline, methane and polymethine, thiazole, indamine and indophenol, azine, oxazine, sulfur, lactone, aminoketone, hydroxyketone, anthraquinone, indigoid, phthalocyanine, and natural which have different colored isomeric form or change color in presence of controller when exposed to steam can be used as indicator.

[0062] A large number of dyes, as listed in Table 1, were explored with and without a variety of additives as indicator controllers in EC001270 as a polymeric binder. Pieces of the coatings were exposed to steam.

[0063] Table 1. List of Dyes and Pigments Tested as an Indicator in EC001270 as a Binder.

[0064] Acid alizarin violet N, acid black 24, acid black 48, acid blue 113, acid blue 120, acid blue 129, acid blue 161, acid blue 25, acid blue 29, acid blue 40, acid blue 41, acid blue 45, acid blue 80, acid blue 93, acid fuschin, acid green 25, acid green 27, acid green 41, acid orange 74, acid red 1, acid red 114, acid red 151, acid red 88, acid violet 17, acid violet 7, acid yellow 99, acridine orange, acridine orange base, acridine orange G, acridine yellow G, acriflavine hydrochloride, alcian blue 8GX, alcian yellow, alizarin, alizarin blue black SN, alizarin complexone, alizarin complexone dihydrate, alizarin red, alizarin violet 3R, alizarin yellow GG, alizarin yellow R, alkali blue 6B, alkali fast green 10GA, alphazurine A, aluminon, aminoacridine hydrochloride, aminoanthraquinone, aminophthalhydrazide, aniline blue, astra blue 6GLL, auramine O, azocarmine, azocarmine B, azure A, azure B, azure B thiocyanate, azure C, basic blue 3, basic blue 41, basic blue 66, basic fuchsin, basic red 29, basic yellow 11, benzo purpurin 4B, biebrich scarlet NA salt, bismarck brown B, bismarck brown Y, blue tetrazolium, bordeaux R, brilliant blue B, brilliant blue G, brilliant cresyl blue ALD, brilliant crocein MOO, brilliant green, brilliant sulphaflavine, brilliant yellow, bromochlorophenol blue, bromocresol green, bromocresol purple, bromophenol blue, bromopyrogallol red, bromothymol blue, bromoxylenol blue, calmagite, carbol fuchsin, carminic acid, carotene, celestine blue, Chicago sky blue, chlorophenol red, chrome azurol S, chromotrope 2B, chromotrope 2R, chromoxane cyanine B, chrysoidin, chrysophenine, cibacron brilliant red 3BA, Congo red, copper(II) phthalocyanine, cresol purple, cresol red, cresol, cresolphthalein, cresolphthalein complexone, crystal violet, curcumin, darrow red, diaminoacridine hemisulfate, diazo red RC, dibromofluorescein, dichlorofluorescein, dichloroindophenol, dicinnamalactone, diethylaminomethyl coumarin, diethyloxacarbocyanine iodide, diethylthiatricarbocyanine iodide, dihydroxy benzenesulfonic acid, dilithium phthalocyanine, dimethyl methylene blue, dimethylglyoxime, dimethylindoaniline, dinitro diphenylamine, diphenylthiocarbazone, direct blue 71, direct green 6, direct red 23, direct red 75, direct red 81, direct violet 51, direct yellow 62, disodium phthalocyanine, disperse blue 14, disperse blue 14, disperse blue 3, disperse orange, disperse orange 11, disperse orange 25, disperse yellow 7, emodin, eosin B, cosin Y, eriochrome black T, eriochrome blue black B, erioglaucine, erythrosin B, ethyl eosin, ethyl orange, ethyl red, ethyl violet, Evans blue, fast black, fast blue B salt, fast blue BB, fast blue RR, fast blue RR salt, fast corinth V salt, fast garnet GBC base, fast green FCF, fast red aluminum salt, fast red violet LB salt, fast violet B salt, fat brown RR fat green GDC salt, flavazin I, fluorescein, fluorexon, gallocyarune, guinea green B, hematoxylin, hydroxy naphthol blue, 1,4-hydroxy-naphthoquinone, indigo, indigo carmine, indoline blue, iron(II) phthalocyanine, janus green B, lacmoid, leishman stain, leuco crystal violet, leucomalachite green, leucoquinizarin, light green SF yellowish, lissamine green B, litmus, luxol fast blue, malachite green base, malachite green hydrochloride, malachite green oxalate, metanill yellow, methyl eosin, methyl green, methyl orange, methyl red, methyl violet 2B, methyl violet B base, methyl yellow, methylene blue, methylene green, methylene violet 3RAX, methylesculetin, methylthymol blue, mordant blue 9, mordant brown 24, mordant brown 4, mordant orange, mordant orange 1, mordant orange 6, mordant red 19, mordant yellow 10, morin hydrate, murexide, naphthochrome green, naphthol AS, naphthol blue black, naphthol green B, naphthol yellow, naphtholbenzein, naphtholbenzene, naphtholphthalein, neutral red, new coccine, new fuchsin, new methylene blue N, nigrosin, Nile blue A, Nile blue chloride, nitrazine yellow, nitro red, nitro-phenanthroline, nitrophenol-2, nitrophenol-3, nitrophenol-4, nitrophenylazo-resorcinol, nuclear fast red, oil blue N, oil red EGN, oil red O, orange G, orange II, palatine chrome black 6BN, palatine fast yellow BLN, pararosaniline acetate, pararosaniline base, pararosaniline chloride, patent blue VF, pentamethoxytriphenylmethanol, phenanthroline, phenazine, phenol red, phenolphthalein, phenolphthalein diphosphate, phenothiazine, phenylazoaniline, phenylazodiphenylamine, phenylazoformic acid, phenylazophenol, phloxine B, phthalocynine, pinacyanol chloride, plasmocorinth, ponceau S, primuline, procion red MX-5B, procion yellow H-E3G, prussian blue, purpurin, pyridlazo naphthol, pyridylazoresorcinol sodium salt, pyrocatechol violet, pyrogallol red, pyronin B, quinaldine red, quinizarin, quinoline yellow, reactive black 5, reactive blue 15, reactive blue 2, reactive blue 4, reactive orange 16, resazurin, resorcin crystal violet, rhodamine B, rhodamine B base, rhodamine GG, rhodamine S. rhodanine, rosalic acid, rose bengal, rose bengal iactone, safranine O, solvent blue 35, solvent blue 59, solvent green 3, styryl 7, sudan black B, sudan orange G, sudan red 7B, sulfobromophthalein sodium salt, sulforhodamine B, tartrazine, tetrabromophenol blue, tetrabromo phenolphthalein, tetrabromo phenolphthalein, tetraiodo phenolphthalein, tetraphenyl-butadiene, tetrazolium violet, thiazol yellow G, thioflavin S, thioflavin T, thionin, thymol blue, thymolphthalein, thymolphthalein monophosphate, thymolphthalein monophosphate, toluidine blue O, triphenylmethyl bromide, tropaelin O, trypan blue, tumeric, vanillin azine, variamine blue RT salt, variamine blue RT salt, victoria blue B, victoria blue B, victoria pure blue BO, wright stain, xilidine ponceau 2R, xylenol blue, and xylenol orange.

[0065] Some of these dyes are fluorescence dyes and there was a change in fluorescence. The indicators which monitors integral value of time, temperature and humidity are often referred to as steam indicators herein.

[0066] A neutral dye or pigment, which does not change color with steam can also be used as an additive to get a series of color changes. For example, addition of a neutral yellow dye or pigment in a dye which changes from colorless to blue with steam (e.g., reduced methylene blue) can provide a series of color change, for example, yellow, yellow-green, green, and blue-green. Similarly, more than one dyes which undergo different color changes, e.g., yellow-to-colorless, red-to-colorless, yellow-red, red-yellow and colorless-to-blue can also be mixed and used to get a series of color change with steam.

[0067] Medical supplies are usually sterilized above 100° C., e.g., for about 20 minutes at 125° C. and 5 minutes at 135° C. In order to use an indicator as a steam sterilization indicator for medical supplies, the indicator preferably must not undergo the color change below 100° C. It must also not undergo color change at high ambient temperature and

humidity. An indicator made from direct blue 71 and EC001270 does not change color at 80° C. for two weeks and under 100% humidity at 80° C. for a week. Commercially available indicators such as those based on lead, change from red to dark brown within two hours at 80° C. at 100% humidity. The preferred indicators for sterilization of medical supplies are direct blue 71, methylene blue, dispersed blue **14** and iron phenanthroline.

[0068] Frozen foods, to be heated either with microwave oven or convention gas or electric ovens, should preferably be heated above at least 80° C, i.e., till some steam is produced. The time required for doneness of the food will depend upon the nature of the food. The indicator to be used for monitoring doneness of food should not change color below about 60° C. An indicator made from methylthymol blue and EC001270 does not change color below 60° C. but change color from red-to-blue in minutes with steam at 80° C. and above. The preferred indicator for doneness of food is methylthymol blue.

[0069] Homes, restaurants and catering organizations use kitchenware such as dishes, cutlery and utensils, which need to be sterilized with either dry heat, hot water and steam usually below 100° C. There is also a need for an indicator, for examples, 90° C. for 10 minutes, to make sure the cookware have been subjected to certain integral value of heat and/or humidity. The preferred indicator for kitchenware is also methylthymol blue.

[0070] If the device undergoes a color change with humidity only, it can be used as a humidity/moisture indicator.

[0071] Any chemical, which can provide a stable isomer of the indicator under ambient conditions and assist or make the indicator to undergo a color change when treated with humidity/steam can be used as an indicator controller. Indicator controllers are also referred to as controllers herein. A controller could also be an isomer or tautomer, oxidizing agent, reducing agent or a solvent. A variety of classes of organic and inorganic compounds can be used as controllers for indicators. They include acetylacetonates, acids, alcohols, aldehydes, amides, amines, azo, bisulfites, bisulfates, carbonates, carbamates, carbazones, chelates, metal complexes, cyanates, esters, halides, halocarbons, imides, imines, ketones, lactams, lactones, mercapto, nitrites, nitrates, nitriles, nitro, nitroso, oximes, pentanediones, phenols, phosphates, sulfates, sulfides, sulfites, thiocyanates, ureas, urethanes, salts, oxidants, reducing agents and solid solvents.

[0072] The specific examples of compounds explored as indicator controllers with some selected dyes (e.g., direct blue 71, methylene blue, and methylthymol blue) are listed in Table 2.

[0073] Table 2. Exemplary Controllers:

[0074] Abietic acid, acetone oxime, aluminum acetylacetonate, aluminum ammonium sulfate, aluminum chloride, aluminum sulfate, amino deoxy d-sorbitol, ammonium acetate, ammonium bisulfite, ammonium bromide, ammonium carbamate, ammonium nitrate, ammonium sulfamate, ammonium sulfite, ammonium thiocyanate, ammonium thiosulfate, ascorbic acid, azodicarbonamide, azodicarbonamide, benzilic acid, benzoic acid, benzophenone, benzophenone tetracarboxylic acid, benzophenonetetracarboxylic diahydride, benzoquinone dioxime, benzoquinone dioxime, benzyloxy)phenol, butyl phenol, caffeine, calcium ferrocyanide, catechol, catechol, chloranilic acid, copper thiocyanate, cupferron, cupferron, cyclopenatanone oxime, dehydroacetic acid, di-butyl-t-4-methylphenol, dihydroxy acetophenone, dihydroxy dimethoxy benzophenone, dihydroxy naphthalein disulfonic acid, dihydroxyacetophenone, dihydroxy-dimethoxybenzophenone, dimethyl fumarate, dimethyl tartrate, diphenyl butyro lactone, diphenylglyoxime, diphenylthiocarbazone, di-t-butyl-4-methylphenol, dithizone or diphenylthiocarbazone, ethylcarbonate, ethylenediamine tetraaceticacid and its salts, ferroin, fumaric acid, gallic acid, gluconic acid fe(ii) salt, glucose penta acetate, glutaaric acid, glycerophosphate, glyconolactone, hexahloro norborene dicarboxylic acid, hydroquinone, hydroxy acetophenone, hydroxy acetophenone, hydroxy cinnamic acid, hydroxy methoxybenzophenone, hydroxy octyloxy benzophenone, hydroxybenzophenone, hydroxymethoxybenzophenone, hydroxyquinoline, hydroxyquinolie, inositol, iron acetylacetonate, iron complexes such as potassium ferrocyanide, iron sulfate, isoascorbic acid, levulinic acid, maleic acid, maleic acid, malic acid, mandelic acid, mercaptobenzothiazole, methyldinitrosalicilate, methyldinitrosalicylate, methylesculetin, methyltrihydroxybenzoate, naphthol, naphthol-disulfonic acid, naphthoquinone tetrasulfate sodium salt, nitron, nitroso-1,2-naphthol, nitrosophenol, oxalic acid, phenanthroline, phthalide, propylgalliate, propylgalliate, pydine aldoxime, pyruvic acid, resorcinol, rutin hydrate, salicyladoxime, salicylanamid, salicylanilide, salicylic acid, sodium acetylacetonate, sodium bisulfite, sodium cyanate, sodium diethyldithiocarbamate, sodium dithionite, sodium hydrosulfide, sodium nitrite, sodium persulfate, sodium sulfite, sodium thiocyanate, sodium thiosulfate, sulfosalicyclic acid 5, tannic acid, tetrabutylphosphonium bromide, tetrahydroxybenzophenone, tetramethylhexane diamine, tetronic acid, tetronic acid, thiodiglycolic acid, thiodipropionic acid, thioglycolic acid, thiourea, tribenzylamine, trichloroacetamide, trichlorobenzylacetate, trihydroxybenzophenone, urea, vitamin-c, and vitamin-c palmitate

[0075] Any chemical, which can provide a stable isomer of the indicator under ambient conditions and assist or make the indicator undergo a color change when treated with humidity/steam is a preferred indicator controller. Preferred class of compounds are those which can form hydrogen bonds, e.g., alcohols, amides, amines, acids, bisulfites, bisulfates, carbonates, carbamates, chelates, metal complexes, cyanates, esters, halides, halocarbons, ketones, nitrites, nitrates, nitriles, nitro, nitroso, oximes, phenols, phosphates, sulfates, sulfides, sulfites, thiocyanates, ureas, and urethanes. The most preferred are hydrogen bond forming controllers are aliphatic and aromatic, primary, secondary and tertiary amines. Examples of amines and their salts include adamantanamine, adenine, amino cyclohexanol, amino diethylaminopentane, amino dodecanoic acid, amino ethyl dihydrogen phosphate, amino ethyl hydrogen sulphate, amino pentenoic acid, amino propyl imidazole, amino propyl pipecoline, amino sorbitol, amino undecanoic acid, amino-butanol, aminodeoxy-d-sorbitol, aminoethyl dihydrogen phosphate, aminopropyl imidizole, ammonium acetate, ammonium bromide, ammonium carbaminate, ammonium carbonate, ammonium chloride, ammonium dibydrogen phosphate, ammonium ferrocyanide hydrate, ammonium formate, ammonium hydrogen carbonate, ammonium hydroxide, ammonium iron(ii) sulfate, ammo-

nium iron(iii) citrate, ammonium iron(iii) oxalate trihydrate, ammonium nitrate, ammonium per sulfate, ammonium phosphate dibasic, ammonium sulfamate, ammonium sulfate, benzyl-n-methylethanolamine, benzyltrimethylammonium chloride, bis(dimethylamino) benzophenone, bis-(diphenylphosphinopropane), butylimidizole, carbonyldiimidazole, carboxycinnamic acid, chloroethyl-trimethyl, chloroethylamine monohydrochloride, chlorohydroxypropyl trimethyl hydrochloride, chloronitroaniline, choline, choline chloride, choline hydroxide, choline iodide, cyclohexyamine, decylamine, diallyl dimethyl ammoniaum chloride, diaminodiphenylamine, diaminododecane, diaminoheptane, diaminohydroxypropane, diaminononane, diaminooxapentane, diaminopropane, dibutylamino propylamine, dibutyl amino benzaldehyde, diethanolamine, diethyl amine, diethyl aminopropylamine, diisopropyl ethylamine, dimethyl amine, dimethyl amino ethylmethylamino ethanol, dimethyl amino benzaldehyde, dimethyl aminopropoxy benzaldehyde, dimethyl aminopropylamine, dimethyl ammopyridine, dimethyl glycine, dimethyl glyoxine, dimethyl imidizole, dimethyl imidizolidinone, dimethyl propane-diamine, diphenylamine, diphenylamine, diphenylbenzidine, dodecylamine, dodecyltrimethylammoniumbromide, ethanolamine, ethanolamine hydrochloride, ethyl amine, ethyl aminobenzoate hydrochloride, glycidil trimethyl ammonium chloride, histidine, hydroxylamine hydrochloride, hydroxylamine sulphate, imidazole, imidazolidone, iminodiacetic acid, methyl amine, methyl imidizole, nitro anilne, nitro diphenylamine, octa decylamine, phenyl enediamine, polyethylenimine, tetrabutyl ammonium hydroxide, tetrabutyl ammonium iodide, tetraethylammonium bromide, tetraethylammonium hydroxide, tetrafluorophenylimidizole, tetrahexylammonium bromide, tetramethyl ammonium acetate, tetramethyl ammonium chloride, tetramethyl ammonium hydroxide, tetramethyl ethylenediamine, tetramethyl ethylethylenediamine, tetramethyl hexanediamine, tetramethyl propanediamine, tetramethyl quanidine, triallylamine, triethanolamine, triethylamine, triethylenetetramine, triethylenetetramine hydrochloride, triethylethylenediamine, triiodecylamine, trimethyl ammonium chloride, trimethyl-propanediamine, trimethylamine hydrochloride, trioctylamine, trioxa-tridecanediamine, triphenylamine, tris(hydroxymethyl) aminomethane, tris(methoxyethoxy) ethylamine. The preferred controller amines are tetramethylhexane diamine, ethanolamine, ethylene diamine and diethylamine.

[0076] The other preferred class of controllers is compounds having ability to isomerize. The preferred isomeric controllers are tautomers. The preferred tautomers are derivatives of CH_3—CO—CH_2—CO—R, e.g., acetoacetic acid and 2,4-pentanedione. The most preferred tautomers are benzylacetoacetate and iron acetylacetonate.

[0077] Another class of controllers is reducing agents. The preferred reducing agents are sodium sulfite, sodium hydrosulfite, sodium borohydride, derivatives of ascorbic acid and hydrazines or mixture thereof.

[0078] Still another class of controllers is oxidizing agents. The preferred oxidizing agents are perchlorates, nitrates and persulfates, e.g., sodium perchlorate, ammonium nitrate, sodium persulfate.

[0079] Concentration of indicator controller required for the noticeable color change depends upon several factors, such as natures of the indicator and controller. Preferred concentration of a controller is 0.1 to 30% of the total solid of the coating. The most preferred range of the controller concentration is 0.5 to 10%.

[0080] A matrix or medium in which the controllers, indicators and any other additives can be dissolved or dispersed are referred herein to as binders, polymers or polymeric binders. A wide variety of polymeric materials can be used as binders for the indicator as long as the controllers and indicators can be dissolved or dispersed in them. Both aqueous and non-aqueous binders can be used. Though one can use water-soluble, water-dispersible and polymers soluble in organic solvent as binders for the indicator, it is desirable to use water-soluble and water-dispersible polymers as binders. The binders can be formulated as ink formulations, such as for use as flexo and gravure inks. Other inks such as those for letter press, offset and screen printing, can also be made and used. Selection of a polymer depends upon the printing/coating equipment to be used.

[0081] As an alternative to the aforesaid binders, one can use ink and coating formulation curable with UV light. UV curable ink and coating formulations include UV polymerizable/curable compounds such as epoxy-acrylate, polyester acrylates, and resins, typically the acrylates of diphenylol propane di-glycidyl ethers, as their principal component. In order to lower viscosity and to provide a bridge between large polymer molecules, acrylic monomers are used, typically the acrylate esters of polyfunctional alcohols or glycols. The use of monomers as crosslinking agents is vital to the rapid formation of cured films, and has a major influence on the properties of both the ink or coating, and the cured product. Printing inks with epoxy-acrylate resins as their main component are usually fast curing. In order to prepare the device, one can dissolve or disperse, the indicator, controller, and additives in the UV curable extender followed by coating on substrate and curing with UV light UV curable inks which can be used as binders for all kinds of indicators including those for ethylene oxide and plasma can also be used for steam sterilization indicators.

[0082] Usually acrylic polymers, emulsion of acrylic polymers, occasionally natural polymers, such as starch, cellulose, lignins and their derivatives are used as binders for inks. Resins are water soluble or emulsifiable through neutralization with basic compounds, such as ammonia and amines. Inks contain a variety of additives to eliminate foaming, dispersion of pigments, Theological modifiers, and slip agents.

[0083] Polymeric binders for inks include homopolymers, copolymers and block-copolymers including those of ethylene acrylic acid, ethylene methacrylic acid, ethylene n-butyl acrylate, and ethylene methyl acrylate. Binders for inks could also be a mixture of homo and copolymers, e.g., those of methylmethacrylate, acrylic acid, styrene, methyl acrylate, other esters and crosslinking agents, such as polyaziridines and divalent metal salts such as zinc hydroxide.

[0084] Commercial sources for suitable polymers for ink formulations include Air products (Allentown, Pa.), Rohm and Haas (Philadelphia, Pa.), S.C. Johnsons and Sons (Racine, Wis.), Witco (Houston, Pa.) and ESI (Valley Stream, N.Y.). Though a large number of polymers are suitable as ink extenders, EC001270 made by Environmen-

tal Inks and Coating Co., Lithicum, Md. which is composed about 40% styrene-acrylic polymers, a few percent ammonium hydroxide, additives, such as a polymeric wax and an antifoaming agent, alcohol and the balance water, has been found very suitable.

[0085] Though aqueous ink or coating formulations are preferred, one can use solvent based coating formulations polymers used in such formulations are cellulose nitrate, carboxymethyl cellulose, polyolefins, polyvinyl chloride, polyurethane, polysilicones and polyepoxy and UV curable ink formulations.

[0086] When all components of the inks are readily soluble in water, one can make an ink for jet ink printer and indicator device can be made using an inkjet printer.

[0087] The sterilization of an article will also depend on diffusion of steam through the binder. Hence, the time required for the color change of the device can be increased by applying a barrier coat or laminating a film on the device. A barrier coat, or topcoat, can preferably be a polymeric material. The preferred barrier coat is a lacquer or an ink without pigment. The barrier coat can be a polymer listed herein. The general classes of polymers suitable for a barrier coat include resins, such as epoxy, phenol-formaldehyde, amino-formaldehyde, polyamides, vinyls, acrylics, polyurethanes, polyesters, water-soluble resins, alkyds, elastomers, waxes and rosins. Preferred material for topcoat is a paraffin wax through which steam can diffuse slowly.

[0088] The device could have more than one indicator layers each containing indicator, controller and binders. In order to get more than one color change at least the indicator should be different in different indicator layers and should undergo different color changes. Both layers do not have to undergo color changes with steam. Even if one layer undergoes a change in color or opacity, the color change of the other can be noticed, especially if the top layer becomes colorless or transparent.

[0089] Indicator can have an optional topcoat or can be laminated with a transparent film The indicator can also be sandwiched between two layers, one preferably clear for viewing color change.

[0090] Desired colors and color changes can be obtained by mixing proper dyes in appropriate amounts. Similarly, the time required for the color change can be varied by using a proper mixture of the indicators, controllers and additives in appropriate amounts. The desired colors and the time required for the color changes can be obtained by selecting a proper mixture of compatible binders, additives and controllers.

[0091] Though the device could be a self-supporting polymer film containing the controller and indicator, it is desirable to prepare the device on a substrate. The device can be made by coating the indicating formulation on a substrate. The substrate could be any solid surface, for example, that made from paper, plastic, ceramic and metal.

[0092] The substrate could be a container, e.g., bag, pouch, can or container lid, for items to be sterilized or food to be cooked. The sterilization indicator can also be prepared in form of stickers, strips and tapes.

[0093] Although any solid substrate having a smooth surface can be used, a preferred substrate is a flexible and transparent plastic film, and natural (cellulose) and synthetic (e.g., spun bonded polyolefins, e.g., Tyvak®) papers. Plastic films, such as polyethylene, polypropylene, polyvinyl chloride, polymethylmethacrylate, polyurethanes, nylons, polyesters, polycarbonates, polyvinyl acetate, cellophane and esters of cellulose can be used as the transparent substrate. Metal foils, such as aluminum can be used. The most preferred substrates are the 5-300 microns thick films of polyethylene terephthalate, cellulose paper and Tyvak®.

[0094] The indicator could be in the form of any shape, e.g., dot, square, rectangle, picture, image and message.

[0095] The indicator can undergo a color change from a very low temperature (e.g., room temperature) to a very high temperature (e.g., 150° C.) of pressurized steam. The preferred temperature for the color change depends upon the application of the indicator. For monitoring doneness of a food and sterilization of kitchenware, the temperature could be between 60° C. and 100° C. For monitoring steam sterilization of canned foods the temperature could vary from 80° C. to 120° C. and that of medical supplies it could vary from 100° C. to 150° C. The preferred temperature range is 80-140° C.

[0096] The time required for the color change can be varied by varying one or more of the following parameters: thickness of the binder and the indicator layer; thickness of the barrier coat; concentration of the controller; concentration of the indicator; concentration of other additives; nature of the binder, nature of the barrier; nature of the controller; nature of the indicator; nature of the additives; and concentration of water vapor.

[0097] The thickness of the indicator and barrier layers may vary from a micron to five hundred microns. The preferred thickness is approximately 1-50 microns and the most preferred thickness is approximately 2-20 microns.

[0098] The concentration of controller may vary from 0.1 to 50 w/w %. The preferred concentration is 1 to 20 w/w % and the most preferred concentration is 2-10 w/w %.

[0099] The concentration of the indicator may vary from 0.1 to 30 w/w %. The preferred concentration is 1 to 10 w/w % and the most preferred concentration is 2-5 w/w %.

[0100] The concentration of additives such as crosslinking agents, plasticizers, stabilizers and UV absorber may vary from 0.1 to 20 w/w %. The preferred concentration is 0.5 to 10 w/w % and the most preferred concentration is 1-5 w/w/%.

[0101] The time required for the color change will depend upon concentration of steam or humidity and the application of the device. For kitchenware water vapor concentration could be as low as 5% and that for steam sterilization of medical supply, one can use completely saturated steam. The time required for the color change will be shorter with higher concentration of steam/humidity and vice versa. Higher humidity increased the time required for the color change. The preferred concentration is saturated steam.

[0102] The classes and specific polymer listed herein can be used and preferred as binder and barrier polymers. Preferred binders are polyacrylates.

[0103] The classes and specific controllers, indicators, and additives listed herein can be used and preferred as controllers, indicators, and additives.

[0104] The preferred time range for the indicator will depend upon the application and the temperature of sterilization. The preferred time for sterilization is from 1 to 100 minutes. The most preferred time is 2 to 30 minutes. The preferred time range for doneness of food and sterilization of kitchenware also depend upon the temperature of the warm up. The preferred time for doneness of the food is from 1 to 100 minutes. The most preferred time is 2 to 15 minutes.

[0105] We have found that most of the formulations reported herein were not effected by ethylene oxide, plasma and normal ambient conditions such as humidity and light.

[0106] The devices described here are integrators, i.e., they monitor integral value of time, temperature and water vapor.

[0107] The device offers many advantages including: the formulations are inexpensive; the ingredients are considered nontoxic; it is easy to make the ink formulations, just by mixing proper ingredients in an ink extender, the device is selective to water vapor and steam; the device is unaffected by ethylene oxide, dry heat and radiation; it is unaffected by sealing hot bar, the ink has required pot life; there is no bleeding/diffusion of dyes; the ingredients (indicators/dyes and controllers/additives) are water soluble; no grinding of ingredients is required; ink is printable with gravure and flexo presses on polyester, paper and type; the print rolls are easy to clean; the time required for the color change can be varied by simple means; and it provides desired color changes (from a starting light color, such as orange, pink, or red to a final dark color, such as blue, green, black, purple or violet).

EXAMPLES

Example 1

[0108] General Procedure for Preparation of the Sample Devices.

[0109] In a 10 ml test tube were added about 25 mg of a controller (e.g., tetramethylhexane diamine) and about 0.5 ml of an indicator solution (e.g., 4 w/w % solutions of direct blue 71 in water). The content is mixed and heated if required. In the mixture was added about 1 g of EC001270. In order to control the diffusion of steam, some times solution of a polyaziridine or ammonical zinc oxide was added. The contents were mixed and coated with #5 or #10 wire wound rod on a 100 micron polyester film and paper. The coatings were dried in an oven at about 50° C. for about a few minutes.

Example 2

[0110] Exposure to Steam and Humidity

[0111] Samples of example 1 were placed in a (1) humidity chamber e.g., 100% humidity at 60 or 70° C. and (2) in a pressure cooker at ~123° C., i.e. at 25 lbs, for different periods. The color changes of the samples were noted. Some samples were exposed to steam at 134° C. for 1 to 6 minutes. In order to determine selectivity, some samples were also exposed to ethylene oxide (e.g., 100% gas for about 3 hours) and dry heat (usually for 16 hours at 70° C.).

Example 3

[0112] Different Dyes and Controllers.

[0113] Using the general procedure described in example 1, coatings were prepared from EC001270 as a binder, tetramethylhexane diamine, aluminum acetylacetonate, sodium acetylacetonate, benzylacetylacetonate, sodium sulfite, ascorbic acid, sodium thiocyanate and tetraethylammonium bromide as controllers, and most of the dyes listed in Table 1 as indicators. The coatings were exposed to steam for 20 minute at 123° C. Some representative color changes are listed Table 3.

TABLE 3

Representative color changes of some dyes with EC001270

Dye	Controller	Original	Steam treated
Direct blue 71	None	Light-blue	Blue
Direct blue 71	TMHDA	Red-purple	Blue
Direct blue 71	SS	Faint blue	Blue
Methylthymol blue	None	Orange	Red
Methyl thymol blue	AAA	Red	Blue
Auramine O	None	Yellow	Colorless
Methylene blue	SS	Light red	Blue

[0114] TMHDA: Tetramethylhexane diamine, AAA: aluminum acetylacetonate, and SS: sodium sulfite

Example 4

[0115] Pilot Coating of Methylthymol Blue as an Indicator and Aluminum Acetylacetonate as a Controller.

[0116] Using the general procedure of example 1, an ink formulation was prepared by mixing 1250 g of EC001270 as binder, 50 g of methylthymol blue [3,3'-bis{N,N-di(carboxymethyl)-aminomethyl}thymolsulfonephthalein] as an indicator and 20 g of aluminum acetylacetonate dissolved in 200 g of methanol as a controller. The mixture was coated on paper and polyester film using a pilot coater of Rexam Medical Packaging, Mt Holly, N.J. The coatings were burgundy red color. When treated with water vapor (steam) above about 60° C., it changed to blue color. The time required for the color change depend upon the temperature of the vapor. The higher the temperature shorter was the time. For example, it changed to blue within a minute at 100° C. while it took about 10 minutes to change at 90° C.

[0117] This indicator can be used for monitoring doneness of microwave food and sterilization of kitchenware.

[0118] Addition of controllers such as aminocaproic acid, dimethylethanolamine, gluconic acid-iron salt, histidine, thiourea, and calcium acetylacetonate varied the time required for the color change.

Example 5

[0119] Direct Blue 71 as Indicator

[0120] Using the general procedure of example 1, an ink formulation was prepared by mixing 1000 g of EC001270 as binder, 20 g of direct blue 71 as an indicator and 20 g of tetramethylhexane diamine as a controller and 25 g of 20% zinc oxide solution. The mixture was coated on paper and polyester film. The coatings were purple color and changed to blue color when exposed to steam.

[0121] A large number of additives, e.g., those listed in Table 2 were added as controllers instead of tetramethyl-

hexane diamine. Many salts and amines were effective controllers. They include benzylmethylethanolamine, cyclohexylamine, 1,12-diaminododecane, 1,5-diaminopropane, dibutylamino propylamine, dibutyl amino-benzaldehyde, diethanolamine, diethyl amine, dimethyl amine, dimethylethanolamine, diphenylamine, ethanolamine, ethylene diamine guanidine carbonate, hexanediamine, hexylamine, histidine, lysine, morpholine, potassium nitrate, sacrosine, sodium chloride, sodium thiocyanate, 1,1,3,3-tetramethyl guanidine, tetraethylammonium hydroxide, tetramethylethylene diamine, triethanolamine, triethylenetetramine, and trihydroxymethylaminomethane and zinc oxide. Certain reducing agents such as ascorbic acid and sodium sulfite as controller provided almost colorless (faint blue coating) coating which turned blue when treated with steam.

Example 6

[0122] Different Colors with Other Dyes.

[0123] In about 1 g indicator ink of formulation of direct blue 71 of example 5 were added about 0.25 ml of 4% solution of the dyes listed in Table 1. The mixtures were coated on paper and polyester. Many dyes provided different starting, intermediate and final colors. Some representative examples are shown in Table 4.

TABLE 4

Some representative examples of color change direct blue 71 and some dyes upon steam treatment.

Added dye	Original color	After steam treatment
Auramine-O	Khaki yellow →	Green-blue
Quinoline yellow	Purple	Green-blue
Rhodamine	Purple	Blue
Bromocresol purple	Purple	Green-blue
pararosaniline acetate	Red	Violet-blue
Brilliant crocein MOO	Red	Blue

Example 7

[0124] Oxidizing Agents as Controllers.

[0125] Using the general procedure described in example 1, coatings were prepared from EC001270 as a binder containing 10% ammonium nitrate as controller and most of the dyes listed in Table 1 as indicators. Perchloric acid and potassium persulfate were also very effective controllers. The coatings were exposed to steam for 20 minute at 123° C. Some representative color changes are listed Table 5.

TABLE 5

Representative examples of color change of some dyes with sodium nitrate upon steam treatment.

Dye	Original color	After steam treatment
Acid red 88	Light red	Red
Acid alizarin violet N	Light violet	Violet
Benzo purpurin 4B	Red	Light red
Chrysophenine	Light yellow	Yellow
Direct red 75	Light red	Red
Acid blue 113	Blue	Purple
Leuco crystal violet	Colorless	Violet
Bromophenol blue	Light blue	Blue
m-Cresol purple	Orange	Yellow
Dimethylmethylene blue	Light blue	Blue

TABLE 5-continued

Representative examples of color change of some dyes with sodium nitrate upon steam treatment.

Dye	Original color	After steam treatment
Pyrogallol red	Purple	Blue
Nile blue chloride	Light blue	Blue
Methylene blue	Light blue	Blue

Example 8

[0126] Solid Solvents as Controllers.

[0127] Coatings were prepared from 1 ml of EC001270 as a binder containing 10% finely milled glucose pentaacetate as a controller and 0.5 ml of 4% solution of most of the dyes listed in Table 1 as indicators. The coatings were exposed to steam for 20 minute at 123° C. Some representative color changes are listed Table 6.

TABLE 6

Representative examples of color change of some dyes with glucose pentaacetate as a controller upon steam treatment.

Dye	Original color	After steam treatment
Chrysoidin	Orange	Yellow
Bismarck brown Y	Orange	Colorless
Congo red	Red	Colorless
Bromopyrogallol red	Purple	Black blue
Nile blue chloride	Light Blue	Blue
Darrow red	Light pink	Yellow
Disperse blue 14	Colorless	Blue
Solvent blue 59	Colorless	Blue
Oil blue N	Colorless	Blue
Solvent green 3	Colorless	Green
Eriochrome blue black B	Red	Purple
Hematoxylin	Light purple	Purple/blue

Example 9

[0128] Reducing Agents as Controllers.

[0129] Using the general procedure described in example 1, coatings were prepared from EC001270 as a binder, sodium sulfite as controller and most of the dyes listed in Table 1 as indicators. The coatings were exposed to steam for 20 minute at 123° C. Some representative color changes are listed in Table 7.

TABLE 7

Representative examples of color change of some dyes with sodium sulfite upon steam treatment.

Dye	Original color	After steam treatment
Janus green B	Purple	Blue
Indoline blue	Red	Blue
Acid blue 93	Light blue	Blue
Brilliant crocein MOO	Yellow	Red
Guinea green B	Colorless	Green
Naphthochrome green	Colorless	Blue
Methylene blue	Colorless	Blue
Thionin	Red	Blue
Leishman stain	Red	Blue

[0130] With the above representative examples, we have demonstrated that many dyes and pigments can be made to undergo a color change with controllers such as amines, salts, oxidizing agents, reducing agents and solid solvents. The color change could be due to isomerization of the dye/pigment molecules, oxidation, reduction, dissolution or combination of them.

[0131] Even though we have given some representative examples of dyes, a large number of other dyes, pigments and their mixtures can be used. Similarly, one can use a mixture of controllers and stabilizers to stabilize the isomorphic forms.

[0132] Clearly, it should now be quite evident to those skilled in the art, that while my invention was shown and described in detail in the context of a preferred embodiment, a wide variety of other modifications and variations can be made without departing from scope of my inventive teachings.

I claim:

1. A device for monitoring integral value of time, temperature and water vapor comprising at least one layer of polymer comprising an isomeric indicator capable of undergoing at least one color change, a controller for said indicator capable of influencing the time and temperature required for said color change when contacted with water vapor, wherein said indicator undergoes an isomerization reaction which causes said indicator to undergo said color change.

2. The device of claim 1 where isomerization is due to phase change, polymorphism, tautomerism, dissolution, oxidation or reduction.

3. The device of claim 2 wherein said indicator has at least two isomeric forms each having different colors.

4. The device of claim 2 where said color change is due to dissolution of said indicator.

5. The device of claim 2 where said color change is due to oxidation of said indicator.

6. The device of claim 2 where said color change is due to reduction of said indicator.

7. The device of claim 1 wherein said indicator comprises at least one member of the group consisting of pigments, dyes, and precursors of dyes and pigments.

8. The device of claim 7 wherein said indicator comprises at least one member chosen from the group consisting of acid alizarin violet N, acid blue 113, acid blue 93, acid red 88, auramine-O, azures, benzo purpurin 4B, bismarck brown Y, brilliant crocein MOO, bromocresol purple, bromophenol blue, bromopyrogallol red, chrysoidin, chrysophenine, Congo red, darrow red, dimethylmethylene blue, direct blue 71, direct red 75, disperse blue 14, eriochrome blue black B, Guinea green B, indoline blue, Janus green B, leuco crystal violet, meta-cresol purple, methylene blue, methythymol blue, naphthochrome green, Nile blue chloride, oil blue N, pararosaniline acetate, pyrogallol red, quinoline yellow, rhodamine, solvent blue 59, solvent green 3, and thionin.

9. The device of claim 1 where said layer comprises said indicator and at least one neutral coloring material which does not change color with humidity.

10. The device of claim 1 wherein said color change is selected from a group consisting of red-to-blue, purple-to-blue, yellow-to-blue, colorless-to-green, colorless-to-blue, colorless-to-red, blue-to-red, red-to-yellow, and green-to-colorless.

11. The device of claim 1 wherein said polymer is soluble in an organic solvent.

12. The device of claim 1 wherein said polymer is soluble in water or is water dispersible.

13. The device of claim 12 wherein said polymer is a water soluble or water dispersable homopolymer, copolymer or a mixture thereof.

14. The device of claim 1 wherein said polymer comprises polymerized monomers of styrene, acrylate, acrylic acid, acrylamide, vinyl acetate, vinyl alcohol, vinyl chloride, polyurethanes, cellulose nitrate and carboxymethyl cellulose.

15. The device of claim 1 wherein said polymer is a homopolymer, copolymer or a mixture thereof.

16. The device of claim 1 wherein said polymer is an acrylate polymer.

17. The device of claim 1 wherein said polymer is cellulose nitrate or carboxymethylcellulose.

18. The device of claim 1 wherein said polymer is formed by high energy radiation.

19. The device of claim 1 said polymer is formed by UV light and electron beam.

20. The device of claim 1 wherein said controller is present in an amount of 0.1 to 50%, by weight.

21. The device of claim 20 where said controller is capable of introducing isomerization.

22. The device of claim 1 where said controller comprises a salt, amine, metal chelate, chelating agent, oxidizing agent, reducing agent or solid solvent.

23. The device of claim 22 where said salt is chosen from a group consisting of sodium chloride, sodium thiocyanate and zinc oxide.

24. The device of claim 22 where said amine is chosen from a group consisting of tetramethylhexane diamine, diethylamine, ethylene diamine, diethanolamine, and cyclohexylamine.

25. The device of claim 22 where said oxidizing agent comprises a compound chosen from a group consisting of nitrates, peroxides, persulfates, perchlorates, per-iodates, peroxyacids, amine-oxides, alkyl nitrates, complexes of halides such as bromine, and oxidized metal salts.

26. The device of claim 22 where said oxidizing agent comprises a compound chosen from a group consisting of ammonium nitrate, hydrogen peroxide and sodium persulfate.

27. The device of claim 22 where said reducing agent is chosen from a group consisting of borohydride, sulfite, sulfide, nitrite, salt of a reduced metal, hydride, hydrosulfite, hydazine, oxime, and unsaturated organic compound.

28. The device of claim 22 wherein said reducing agent is chosen from a group consisting of ascorbic acid, sodium sulfite, sodium hydrosulfite, sodium borohydride, sodium nitrite, hydrazine or its derivatives, ammonium sulfite, ammonium thiocyanate, calcium ferrocyanide, Fe(II) salts, isoascorbic acid, sodium bisulfite, sodium cyanate, sodium dithionite, sodium hydrosulfide, sodium sulfite, sodium thiocyanate, sodium thiosulfate, benzoquinone dioxime, cupferron, cyclopenatanone oxime, diphenylglyoxime, salicyladoxime, ascorbic acid and a derivative of ascorbid acid.

29. The device of claim 22 where said chelating agent comprises a compound chosen from a group consisting of benzylacetylacetonate, iron acetylacetonate, and aluminum acetylacetonate.

30. The device of claim 22 where said solid solvent melts below 150° C. or is dissolved with water vapor.

31. The device of claim 22 where said solid solvent comprises a compound chosen from a group consisting of phenol, polyalcohols, acids, amines, esters, amides, lactones, parafins and halogenated parafins.

32. The device of claim 22 where said solid solvent comprises a compound chosen from a group consisting of benzoic acid, diphenyl butyro lactone, glucose pentaacetate, glyconolactone, inositol, chlorinated paraffins, trichlorobenzylacetate, trichloroacetamide, vitamin-c palmitate, tribenzylamine, salicylanamide, and hexachloro norborene dicarboxylic acid.

33. The device of claim 1 wherein the said controller is capable of influencing said time and said temperature required for said color change.

34. The device of claim 1 further comprising a second indicator layer.

35. The device of claim 1 further comprising a polymeric top layer.

36. The device of claim 1 further comprising a wedge shaped polymeric top layer.

37. The device of claim 1 further comprising plurality of devices wherein said plural devices comprise plural indicators and undergo color changes independently.

38. A process of making the device of any of claims 1-37 comprising:

a) dissolving or dispersing said indicator in a solvent to form a solution;

b) applying said solution to a substrate;

c) permitting said solvent to evaporate.

39. The process of claim 38 wherein said substrate is a container for an item to be sterilized.

40. The process of claim 38 wherein said substrate is chosen from plastic film, paper and metal.

41. The process of claim 38 wherein said substrate comprises polyester film, paper or spun bonded polyolefins.

42. The process of claim 38 wherein said solution is an ink formulation.

43. The process of claim 42 wherein said solution is an aqueous ink formulation.

44. The process of claim 42 wherein said ink formulation comprises an acrylate polymer.

45. The process of claim 38 wherein said solution is applied to said substrate by a spraying.

46. The process of claim 45 wherein said spraying is a jet spray.

47. A process of using a device of any of claims 1-37 for monitoring sterilization of materials comprising the steps of:

a) affixing said device to said materials or containers containing said materials;

b) carrying out a process of sterilization;

c) introducing steam during said process of sterilization; and

d) observing a color change indicating said sterilization has proceeded.

48. The process of claim 47 wherein said material is a medical supply, a food, a pharmaceutical, or a biological waste.

49. A process of using the device of claim 1 for monitoring steam comprising the steps of:

a) exposing said device to steam,

b) observing a color change in said device.

50. A formulation for making the device of claim 1 comprising a polymeric binder, indicator, controller and solvent.

51. The formulation of claim 50 further comprising a polymer of any of claims 11-19.

52. The device of claim 1 comprising 50-100%, by weight, of said isomeric indicator.

* * * * *

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(54) VISUAL INDICATING DEVICE FOR BAD BREATH

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(57) ABSTRACT

The invention provides a breath testing device which includes a visual indicating agent which changes color in the presence of an odor associated with bad breath, such as sulfur and ammonia odors. An example of the visual indicating agent is 4,4'-bis(dimethylamino)-benzhydrol (Michler's hydrol or BDMB) and related dyes having a similar chemical structure. The indicating agent is applied to a substrate which is then inserted into a tube or straw, or which covers one end of a straw. When a user with bad breath blows into the tube or straw, the indicating agent will change color. The breath testing devices provide a quick and affordable means for a user to test their breath, and they may be packaged in discreet, pocket-sized dispensers which can be carried in a pocket or purse.

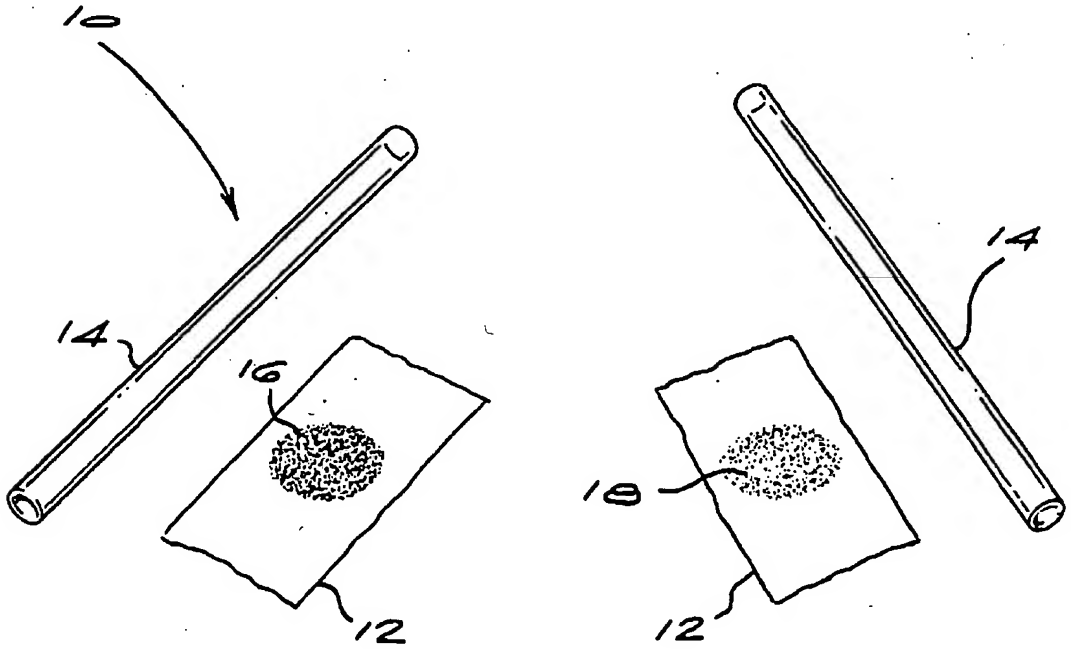


TABLE 6-continued

Results from a test to determine the existence of bad breath using the breath testing article described above

		Results			
				False Assumption[c]	
Volunteer Code	# Tested Samples	True Positive[a]	True Negative[b]	False Positive[d]	False Negative[e]
J	3	2	—	1	—
K	2	1	—	—	1
L	3	3	—	—	—
Total	32	23	2	7	1

KEY:
[a]True Positive = Color change occurred when the user had bad breath.
[b]True Negative = No color change occurred when the user did not have bad breath.
[c]False Assumption = False assumption of the user.
[d]False Positive = User did not believe they had bad breath when in fact they did.
[e]False Negative = No color change occurred when user believed they had bad breath.

[0088] These results indicate that the bad breath visual indicator devices are very sensitive and accurately inform the user of the presence of bad breath. Of the 32 devices tested, 22 showed a clear visual color change when the user thought they had bad breath. Interestingly, 7 color changes were recorded when the user did not think they had bad breath, but in fact they had bad breath. This finding matches the literature percentage of people who cannot detect their own bad breath: 22% of people in this study were unaware that they had bad breath, and the literature reports 25%. Only one breath testing device did not show a change in color when the user believed that they had bad breath.

EXAMPLE 10

[0089] While the breath testing devices described in the previous examples were shown to be suitable for indicating the existence of bad breath, they were still believed to be slightly too large in size to be easily portable and to be able to be carried around discreetly. Further modifications to the breath testing devices were therefore made to miniaturize them further, in particular so that they were small enough so that several could fit into thin breath strip containers which could be carried in a pocket or handbag.

[0090] Two miniaturized breath testing devices were therefore developed:

[0091] a) The first breath testing device (**FIG. 7**) was prepared by taking the article of example 8 (i.e. a drinking straw **40** into which a dye-treated tissue **42** had been inserted) and making the tube of the drinking straw shorter, for example, 4 cm long. The straw was then placed into a standard business card heated laminator (from Kinko's of Dallas, Tex.) so that the tube was flattened but air was still able to pass through the tube.

[0092] b) The second breath testing device (**FIG. 6**) was prepared by taking the article of example 9 (i.e. a drinking straw **44** having a dye-treated tissue strip **46** covering one end), and again cutting it to a length of approximately 4 cm. The straw was laminated as above.

EXAMPLE 11

[0093] A dye-coated paper towel was attached to a 25 mm×50 mm strip of an adhesive-coated card material. The dye content on the paper towel varied depending on the sensitivity required for bad breath testing. Accordingly, 1 mg/ml stock solution of MH-dye was applied on a Snowtex®-O nanoparticle-coated Scott® paper towel and allowed to air dry, before being attached to the strip. A small straw was also placed onto the card and the device was packaged in a polyethylene film cover and the edges were heat sealed. A removable, peal-back polyethylene tab was then used to cover and temporarily seal the dye-coated paper towel. When the tab was pealed back and a user with bad breath exhaled onto the paper towel, the MH-dye turned colorless.

EXAMPLE 12

[0094] Suitable dispensers for the breath testing devices were designed. These dispensers were relatively small, so that they could discreetly fit into a pocket, purse or handbag. Dispensers should also be inexpensive as the breath testing devices are intended to be disposable.

[0095] A small plastic rectangular dispenser (approximately 35 mm×60 mm×15 mm) which can hold about six breath testing devices of the type described in Example 10 was designed (**FIG. 8**), and a slightly larger dispenser for holding **12** breath testing articles is also envisaged. However, it should be clear that a dispenser could be sized to store any number of breath testing articles.

[0096] The dispenser **50** of this example was designed to be attachable to another dispenser **52** for breath mints, chewing gum, cigarettes or the like (**FIG. 9**). The dispensers are permanently attached to each other by means of a glue or double-sided tape in one embodiment. In an alternative embodiment, the dispenser **52** for the breath testing articles may have temporary attachment means, such as a magnet **54**, or hook and loop fasteners (not shown) and can be attached to the second dispenser **52** as shown in **FIG. 9**, by means of an oppositely polarized magnet **56** attached to the other dispenser **52**. Thus, the dispensers can be sold individually so that only one may be replaced at a time.

[0097] While the invention has been described in detail with respect to specific embodiments thereof, it will be apparent to those skilled in the art that various alterations, modifications and other changes may be made to the invention without departing from the spirit and scope of the present invention. It is therefore intended that the claims cover or encompass all such modifications, alterations and/or changes.

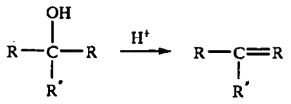
1-22. (canceled)

23. A breath testing device comprising nanoparticles and a visual indicating agent that is color sensitive to at least one odorous compound present in the breath of a user.

24. The breath testing device of claim 23, wherein the odorous compound contains sulfur.

25. The breath testing device of claim 23, wherein the odorous compound contains an amine.

26. The breath testing device of claim 23, wherein the visual indicating agent contains a dye having the general formula (I) or (II):



(I) (II)

where,

R is H, $(NH_2)C_6H_5$—, or C_6H_5—;

R' is $(CH_3)_2NC_6H_5$—, $(NH_2)C_6H_5$—, $(CH_3)C_{10}H_6(OH)$—, or $(NaCO_2)(CH_3)C_{10}H_5(OH)$—; and

R" is $(CH_3)_2NC_6H_5$—, $(NH_2)C_6H_5$—, $(CH_2)C_{10}H_6O$—, or $(NaCO_2)(CH_2)C_{10}H_5O$—;

27. The breath testing device of claim 23, wherein the visual indicating agent contains pararosaniline base, alpha-naphtholbenzein, napthochrome green, or combinations thereof.

28. The breath testing device of claim 23, wherein the visual indicating agent contains 4,4'-bis(dimethylamino)-benzhydrol.

29. The breath testing device of claim 23, wherein the nanoparticles have an average size of less than about 100 nanometers.

30. The breath testing device of claim 23, wherein the nanoparticles have an average size of from about 1 to about 50 nanometers.

31. The breath testing device of claim 23, wherein the nanoparticles have a surface area of from about 50 to about 1000 square meters per gram.

32. The breath testing device of claim 23, wherein the nanoparticles have an average size of from about 100 to about 600 square meters per gram.

33. The breath testing device of claim 23, wherein the nanoparticles include silica, alumina, or combinations thereof.

34. The breath testing device of claim 23, wherein the visual indicating agent is contained on a substrate.

35. The breath testing device of claim 34, wherein the substrate contains a fibrous material.

36. The breath testing device of claim 35, wherein the fibrous material contains cellulosic fibers.

37. The breath testing device of claim 34, wherein the substrate is located within a passage of a carrier portion.

38. The breath testing device of claim 34, wherein the substrate covers an end of a carrier portion.

39. The breath testing device of claim 34, wherein the visual indicating agent is applied to the substrate as a solution.

40. The breath testing device of claim 39, wherein the concentration of the visual indicating agent is from about 0.001 to about 15% wt/wt.

41. The breath testing device of claim 39, wherein the concentration of the visual indicating agent is from about 0.005 to about 2% wt/wt.

42. The breath testing device of claim 23, further comprising a zone having a reference color, the reference color being the color to which the indicating agent will change upon exposure to the odorous compound.

43. A dispenser containing the breath testing device of claim 1.

44. The dispenser of claim 43, further comprising at least one breath freshener.

45. The dispenser of claim 44, wherein the breath testing device and breath freshener are contained in different compartments of the dispenser.

46. A breath testing device comprising a carrier portion defining a passage that is open at least one end, wherein the device contains nanoparticles and a visual indicating agent that is color sensitive to at least one odorous compound present in the breath of a user.

47. The breath testing device of claim 46, wherein the carrier portion is a cylindrical structure.

48. The breath testing device of claim 46, wherein the carrier portion is substantially flattened.

49. A method for testing for bad breath in a user, the method comprising:

causing the user to blow or breathe onto or into a carrier portion of a breath testing device, the breath testing device containing nanoparticles and a visual indicating agent that is sensitive to at least one odorous compound; and

observing whether the visual indicating agent changes color.

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DOCUMENT-IDENTIFIER: US 20050085739 A1
TITLE: Visual indicating device for bad breath

Abstract Paragraph:
The invention provides a breath testing device which includes a visual indicating agent which changes color in the presence of an odor associated with bad breath, such as sulfur and ammonia odors. An example of the visual indicating agent is 4,4'-bis(dimethylamino)-benzhydrol (Michler's hydrol or BDMB) and related dyes having a similar chemical structure. The indicating agent is applied to a substrate which is then inserted into a tube or straw, or which covers one end of a straw. When a user with bad breath blows into the tube or straw, the indicating agent will change color. The breath testing devices provide a quick and affordable means for a user to test their breath, and they may be packaged in discreet, pocket-sized dispensers which can be carried in a pocket or purse.

Pre-Grant Publication (PGPub) Document Number:
20050085739

Detail Description Paragraph:
[0049] According to another embodiment, the breath testing devices described above were made smaller in size so that several of the breath testing devices could be easily packaged together in a pocket-sized container. A straw was cut to about 4 cm in length, and a dye-treated substrate was either inserted into the straw or was placed over one end of the straw. The straw was then substantially flattened by laminating it in a standard business card heated laminator so that air was still able to pass through the tube of the straw.

Detail Description Paragraph:
[0093] A dye-coated paper towel was attached to a 25 mm.times.50 mm strip of an adhesive-coated card material. The dye content on the paper towel varied depending on the sensitivity required for bad breath testing. Accordingly, 1 mg/ml stock solution of MH-dye was applied on a Snowtex.RTM.-O nanoparticle-coated Scott.RTM. paper towel and allowed to air dry, before being attached to the strip. A small straw was also placed onto the card and the device was packaged in a polyethylene film cover and the edges were heat sealed. A removable, peal-back polyethylene tab was then used to cover and temporarily seal the dye-coated paper towel. When the tab was pealed back and a user with bad breath exhaled onto the paper towel, the MH-dye turned colorless.

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Boga et al. (43) **Pub. Date: Sep. 1, 2005**

(54) **ASSAY DEVICES UTILIZING CHEMICHROMIC DYES**

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(52) **U.S. Cl.** **435/7.1**; 435/34

(57) **ABSTRACT**

An assay device for detecting amines within a test sample (e.g., vaginal fluid) is provided. The assay device comprises a detection zone within which a chemichromic dye is contained. The chemichromic dye is capable of undergoing a color change upon exposure to one or more amines within the test sample.

The assay devices were visually observed for the detection signal intensity. For Sample 3, the chemichromic dye remained an orange color and the CRP-detection zone exhibited no color. For Sample 2, the chemichromic dye changed from an orange color to a gray color, but the CRP-detection zone still exhibited no color. Finally, for Sample 1, the chemichromic dye changed from an orange color to a gray color and the CRP-detection zone exhibited a red color.

EXAMPLE 5

[0072] The ability to detect an amine using a lateral flow assay device was demonstrated. Assay device samples were prepared as described above in Example 4. Thereafter, solutions were provided that contained varying concentrations of putrescine (Sigma-Aldrich Chemical Company of Milwaukee, Wis., USA, 99% pure) in acetonitrile, i.e., 0.0, 0.15, 0.30, 0.60, 1.25, 2.50, 5.00, and 10.00 milligrams of putrescine per milliliter. These solutions were applied to the samples, and reflectance readings were then measured for the samples as shown in **FIG. 7**. As indicated, the dye readily detected the presence of putrescine. Further, the level of detection sensitivity was readily controlled by varying the dye concentration.

[0073] While the invention has been described in detail with respect to the specific embodiments thereof, it will be appreciated that those skilled in the art, upon attaining an understanding of the foregoing, may readily conceive of alterations to, variations of, and equivalents to these embodiments. Accordingly, the scope of the present invention should be assessed as that of the appended claims and any equivalents thereto.

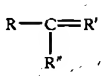
What is claimed is:

1. An assay device for detecting the presence or absence of amines within a test sample, said assay device comprising a fluidic medium that defines a detection zone, wherein a chemichromic dye is contained within said detection zone, said chemichromic dye being capable of undergoing a detectable color change upon reaction with one or more amines.

2. An assay device as defined in claim 1, wherein said chemichromic dye is an arylmethane.

3. An assay device as defined in claim 2, wherein said arylmethane is selected from the group consisting of diarylmethanes and triarylmethanes.

4. An assay device as defined in claim 2, wherein said chemichromic dye is a triarylmethane having the following general structure:



wherein R, R', and R" are independently selected from substituted and unsubstituted aryl groups.

5. An assay device as defined in claim 4, wherein said aryl groups are phenyl groups, naphthyl groups, or anthracenyl groups.

6. An assay device as defined in claim 5, wherein at least one of said aryl groups is amino-substituted, hydroxyl-substituted, carboxyl-substituted, sulfonic-substituted, alkyl-substituted, carbonyl-substituted, or combinations thereof.

7. An assay device as defined in claim 4, wherein said triarylmethane is pararosanilin, alpha-naphtholbenzein, naphthocrome green, or analogs thereof.

8. An assay device as defined in claim 3, wherein said chemichromic dye is a diarylmethane.

9. An assay device as defined in claim 8, wherein said diarylmethane is 4,4'-bis (dimethylamino) benzhydrol or analogs thereof.

10. An assay device as defined in claim 1, wherein said fluidic medium is a porous membrane.

11. An assay device as defined in claim 1, wherein said fluidic medium includes at least one flow channel.

12. An assay device as defined in claim 1, wherein said fluidic medium is in fluid communication with detection probes.

13. An assay device as defined in claim 12, wherein said detection probes are conjugated with a specific binding member for the analyte.

14. An assay device as defined in claim 13, wherein said fluidic medium defines a second detection zone within which is immobilized a capture reagent, said capture reagent being configured to bind to said detection probes or complexes thereof to generate a detection signal, wherein the amount of an analyte in the test sample is proportional to the intensity of said detection signal.

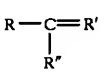
15. An assay device as defined in claim 1, wherein said fluidic medium further defines a control zone within which a chemichromic dye is contained, said control zone being located downstream from said detection zone.

16. An assay device for detecting the presence or absence of both amines and an analyte within a test sample, said assay device comprising a porous membrane that is in fluid communication with detection probes conjugated with a specific binding for the analyte, said porous membrane defining:

a first detection zone within which a triarylmethane dye is immobilized, said triarylmethane dye being capable of undergoing a detectable color change upon reaction with one or more amines; and

a second detection zone within which a capture reagent is immobilized, said capture reagent being configured to bind to said detection probes or complexes thereof to generate a detection signal, wherein the amount of an analyte in the test sample is proportional to the intensity of said detection signal.

17. An assay device as defined in claim 16, wherein said triarylmethane has the following general structure:



wherein R, R', and R" are independently selected from substituted and unsubstituted aryl groups.

18. An assay device as defined in claim 17, wherein said aryl groups are phenyl groups, naphthyl groups, or anthracenyl groups.

19. An assay device as defined in claim 18, wherein at least one of said aryl groups is amino-substituted, hydroxyl-

substituted, carboxyl-substituted, alkyl-substituted, sulfonic-substituted, carbonyl-substituted, or combinations thereof.

20. An assay device as defined in claim 16, wherein said triarylmethane is pararosanilin, alpha-naphtholbenzein, naphthocrome green, or analogs thereof.

21. An assay device as defined in claim 16, wherein said porous membrane further defines a control zone within which a chemichromic dye is contained, said control zone being located downstream from said detection zone.

22. A method for detecting the presence or absence of amines within a test sample, said method comprising:

i) contacting an assay device with a test sample containing one or more amines, said assay device comprising a fluidic medium that defines a detection zone, wherein a chemichromic dye is contained within said detection zone that undergoes a color change upon reacting with said amines; and

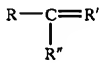
ii) measuring the color intensity of said chemichromic dye at said detection zone after reacting with said amines, wherein said color intensity corresponds to a certain concentration of said amines within the test sample.

23. A method as defined in claim 22, further comprising comparing the measured color intensity with a color intensity of a chemichromic dye that is not reacted with amines.

24. A method as defined in claim 23, wherein said chemichromic dye that is not reacted with amines is contained within a control zone, said control zone being defined by said fluidic medium and being located downstream from said detection zone.

25. A method as defined in claim 22, wherein said chemichromic dye is an arylmethane.

26. A method as defined in claim 25, wherein said chemichromic dye is a triarylmethane having the following general structure:



wherein R, R', and R" are independently selected from substituted and unsubstituted phenyl groups, naphthyl groups, and anthracenyl groups.

27. A method as defined in claim 26, wherein at least one of R, R', or R" is amino-substituted, hydroxyl-substituted, carboxyl-substituted, alkyl-substituted, carbonyl-substituted, sulfonic-substituted, or combinations thereof.

28. A method as defined in claim 26, wherein said triarylmethane is pararosanilin, alpha-naphtholbenzein, naphthocrome green, or analogs thereof.

29. A method as defined in claim 22, wherein said fluidic medium is a porous membrane.

30. A method as defined in claim 22, wherein said fluidic medium is in fluid communication with detection probes conjugated with a specific binding member for the analyte.

31. A method as defined in claim 22, wherein said fluidic medium defines a second detection zone within which a capture reagent is immobilized, said second detection zone being configured to generate a detection signal.

32. A method as defined in claim 31, further comprising measuring the intensity of said detection signal, wherein the amount of an analyte in the test sample is proportional to the intensity of said detection signal.

33. A method as defined in claim 32, wherein said fluidic medium defines a calibration zone that is configured to generate a calibration signal.

34. A method as defined in claim 33, further comprising calibrating the intensity of said detection signal with the intensity of said calibration signal.

35. A method as defined in claim 22, wherein the presence of said amines in the test sample reflects the presence of infection.

36. A method as defined in claim 35, wherein the test sample is obtained from vaginal fluid.

37. A method as defined in claim 35, wherein the test sample is obtained from a wound exudate.

38. A method as defined in claim 35, wherein the test sample is obtained from food.

* * * * *

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(19) **United States**

(12) **Patent Application Publication** (10) **Pub. No.: US 2005/0112085 A1**

MacDonald et al. (43) **Pub. Date: May 26, 2005**

(54) **ODOR CONTROLLING ARTICLE INCLUDING A VISUAL INDICATING DEVICE FOR MONITORING ODOR ABSORPTION**

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(21) Appl. No.: **10/687,269**

(22) Filed: **Oct. 16, 2003**

Publication Classification

(51) **Int. Cl.**[7] **A61L 9/01**

(52) **U.S. Cl.** **424/76.1**

(57) **ABSTRACT**

The present invention relates to a visual indicating device and an article for controlling odors, in particular foot, garbage, basement, cooking, pet, tobacco, feces and urine odors. The article comprises a visual indicating agent that is color sensitive to the odor, and optionally, an odor absorbing agent. The visual indicating agent changes color when the article has been exposed to a sufficient amount of odor to saturate the article. The indicating agent may be applied in differing concentrations to two or more zones so as to indicate to a user of the article how much of the odor absorbing capacity has been used, or conversely, how much of the odor absorbing capacity remains. Suitable visual indicating agents that change color in response to odors are also described. The article for controlling odors may be a disposable odor absorbing sheet, air freshening product, diaper, undergarment pad, face mask, air filtration device, sanitary napkin, tampon, panty shield or incontinence pad.

apparent to those skilled in the art that various alterations, modifications and other changes may be made to the invention without departing from the spirit and scope of the present invention. It is therefore intended that the claims cover or encompass all such modifications, alterations and/or changes.

What is claimed is:

1. An article for controlling odor, the article comprising at least one visual indicating agent that is color sensitive to the odor.

2. The article of claim 1, which further comprises an odor absorbing agent.

3. The article of claim 1, wherein the visual indicating agent is also an odor absorbing agent.

4. The article of claim 1, wherein the indicating agent indicates when the article has been exposed to sufficient odor to saturate the article.

5. The article of claim 1, wherein the indicating agent is located on an indicating device wherein said device is selected from the group consisting of discs, patches and strips, which is applied to or inserted into the article.

6. The article of claim 1, wherein the indicating agent is printed in solution onto the article and allowed to dry so that the dried residue of the solution remains on the article.

7. The article of claim 1, wherein the indicating agent is coated in solution onto the article and allowed to dry so that the dried residue of the solution remains on the article.

8. The article of claim 1, wherein the indicating agent is applied in differing concentrations in two or more zones to indicate how much of the odor absorbing capacity of the article has not been utilized.

9. The article of claim 1, wherein the indicating agent is applied in differing concentrations in two or more zones to indicate how much of the odor absorbing capacity of the article has been used.

10. The article of claim 1, wherein the odor is selected from the group consisting of body odor, foot odor, urinary odor, tobacco odor, meat odor, garbage odor, basement odor, mercaptans, sulfide, hydrogen sulfide, amines, ammonia, sulfur, sulfur degradation products, aliphatic acids, isovaleric acid, butyric acid and acetic acid.

11. The article of claim 1, wherein the visual indicating agent is selected from the group consisting of neutral red, 3-nitrophenol, Brilliant Yellow, chlorophenol red, Rose Bengal dye, D&C Red 28 dye, 4,4'-bis(dimethylamino)-benzhydrol (BDMD or Michler's hydrol), parosaniline base, alpha-naphtholbenzene, naphthochrome green, methyl red, methyl violet, methyl orange, bromocresol mauve, Acid Blue 80, blue dye Calcocid Blue 2G, ethyl red, bromophenol blue, bromocresol green, crystal violet, cresol red, thymol blue, erythrosine B, 2,4-dinitrophenol, alizarin, bromothymol blue, phenol red, m-nitrophenol, o-cresolphthalein, thymolphthalein, alizarin Yellow Reller, cobalt salts and complexes, copper salts and complexes, copper phenanthroline complexes and iron salts and complexes.

12. The article of claim 11, wherein the visual indicating agent is 4,4'-bis(dimethylamino)-benzhydrol.

13. The article of claim 3, wherein both the odor absorbing agent and visual indicating agent are 4,4'-bis(dimethylamino)-benzhydrol.

14. The article of claim 1, which is selected from a disposable odor absorbing sheet, diaper, undergarment pad, face mask, filtration device, sanitary napkin, tampon, panty shield and incontinence pad.

15. An article for controlling odor comprising a nanoparticle selected from the group consisting of silica, alumina, magnesium oxide, titanium dioxide, iron oxide, gold, zinc oxide, copper oxide, and combinations thereof, having thereon at least one metal ion selected from the group consisting of copper ion, silver ion, gold ion, permanganate ion, chlorite ion, persulfate ion, iron ion, and combinations thereof.

16. A visual indicating device for indicating the ability of an odor absorbing article to absorb odor, which includes at least one zone of a visual indicating agent that changes color when exposed to the odor, said zone having a concentration of the visual indicating agent that changes color to indicate that the article is saturated and should be replaced.

17. A method for visually indicating when an article for controlling odor is saturated comprising the steps of:

introducing into or onto the article a visual indicating agent that is color sensitive to the odor, and

observing the change in color of the indicating agent when the article is saturated with the odor.

18. The use of a visual indicating agent on an article for controlling odor, which provides an indication of when the article is saturated with the odor.

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(54) PERSONAL CARE PRODUCTS WITH VISUAL INDICATOR OF VAGINITIS

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(73) Assignee: Kimberly-Clark Worldwide, Inc.

(21) Appl. No.: 10/961,676

(22) Filed: Oct. 8, 2004

Related U.S. Application Data

(63) Continuation-in-part of application No. 10/729,811, filed on Dec. 5, 2003, now abandoned.

Publication Classification

(51) Int. Cl.[7] G01N 33/00

(52) U.S. Cl. 436/111

(57) ABSTRACT

There is provided a personal care product having a body side liner, a baffle and an indicator strip with two ends. The indicator has an amine sensitive dye near at least one end. The indicator extends from the target area just below the liner to just above the baffle such that the dye deposit is visible to an unaided eye. The dye changes color in the presence of amines which are characteristic of infection, thus alerting the user to the possibility of infection. Such an indicator placed in a feminine hygiene pad, for example, may be useful in the diagnosis of vaginitis.

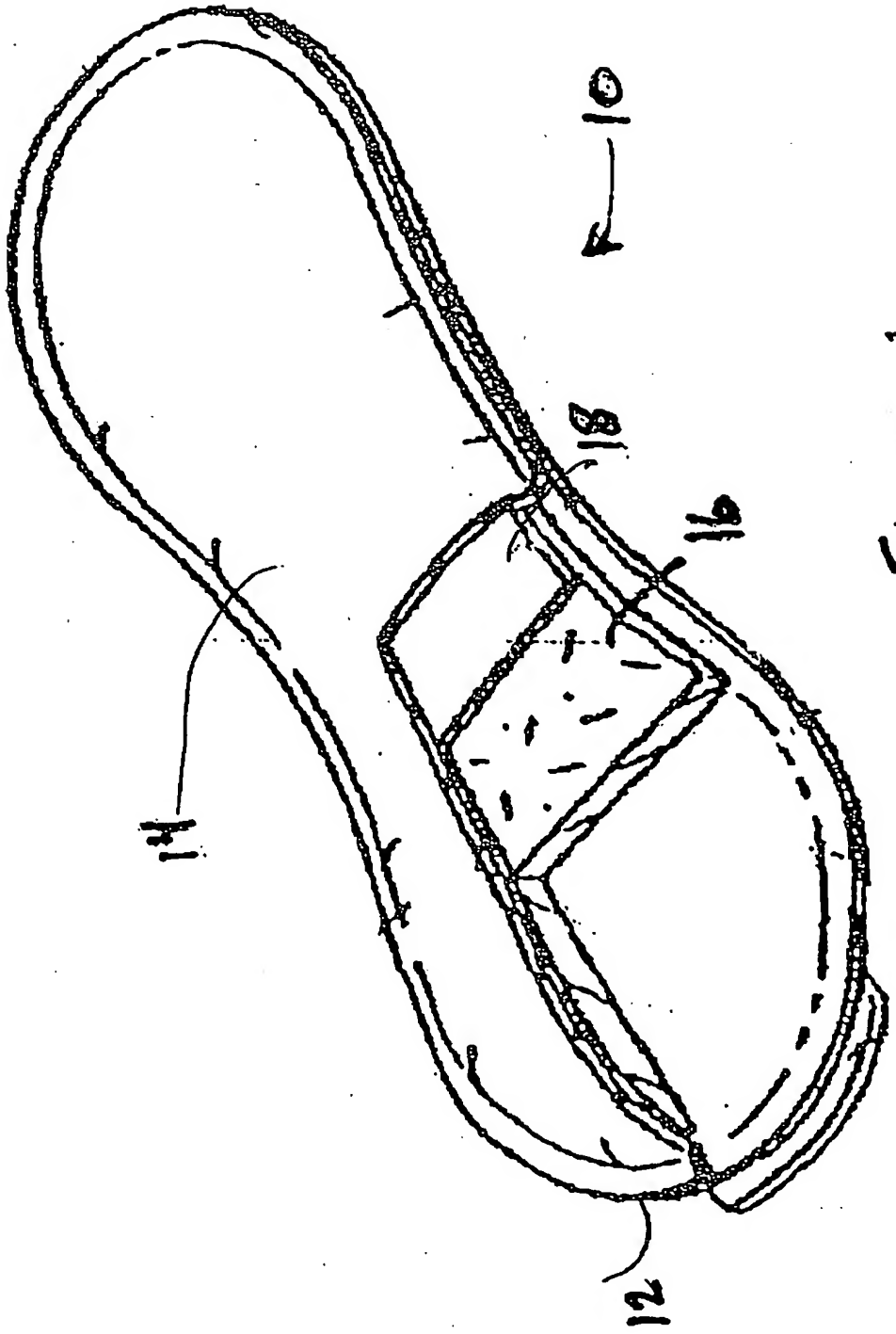


Figure 1

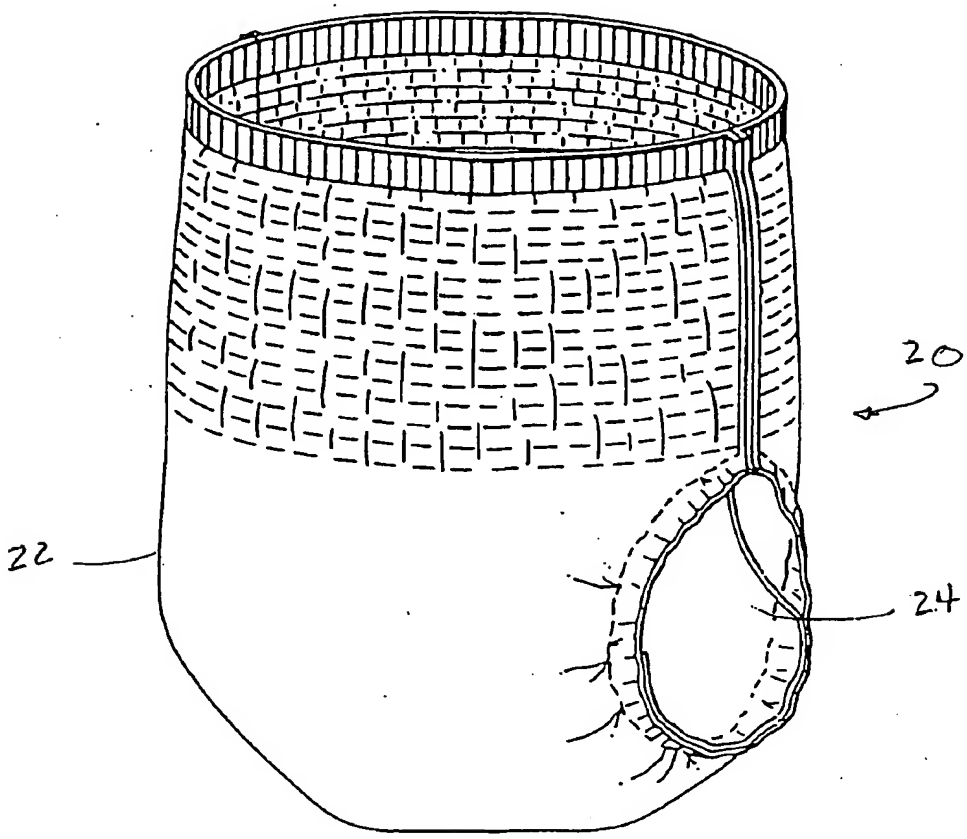


FIG. 2

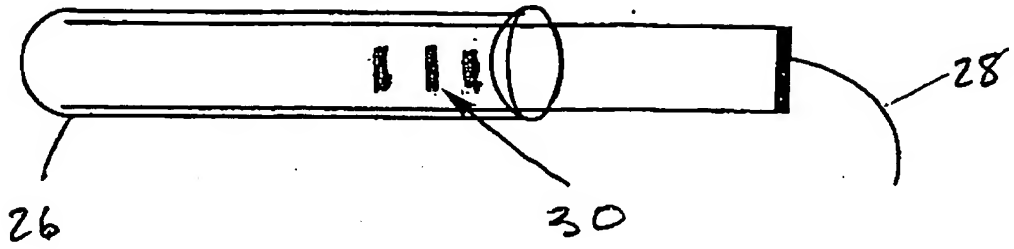


Figure 3

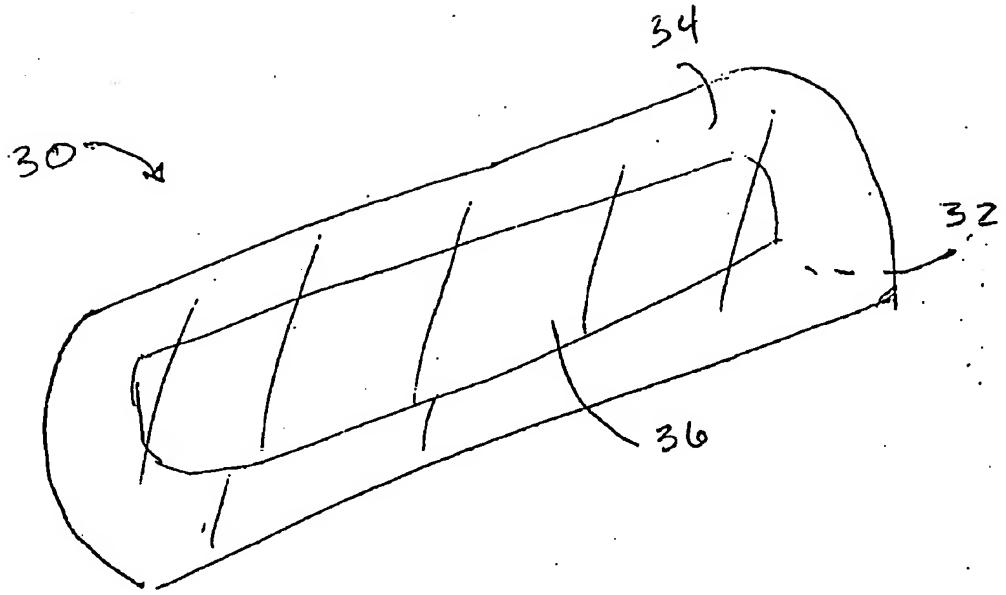
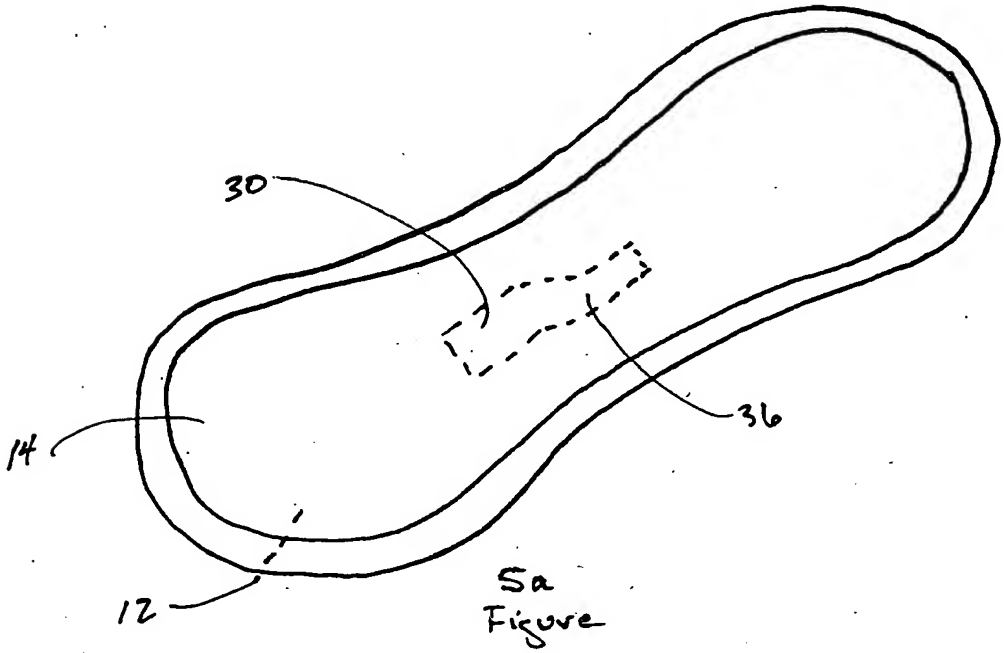
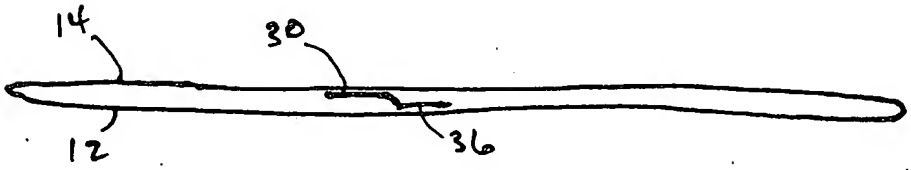


Figure 4



5a Figure



5b Figure

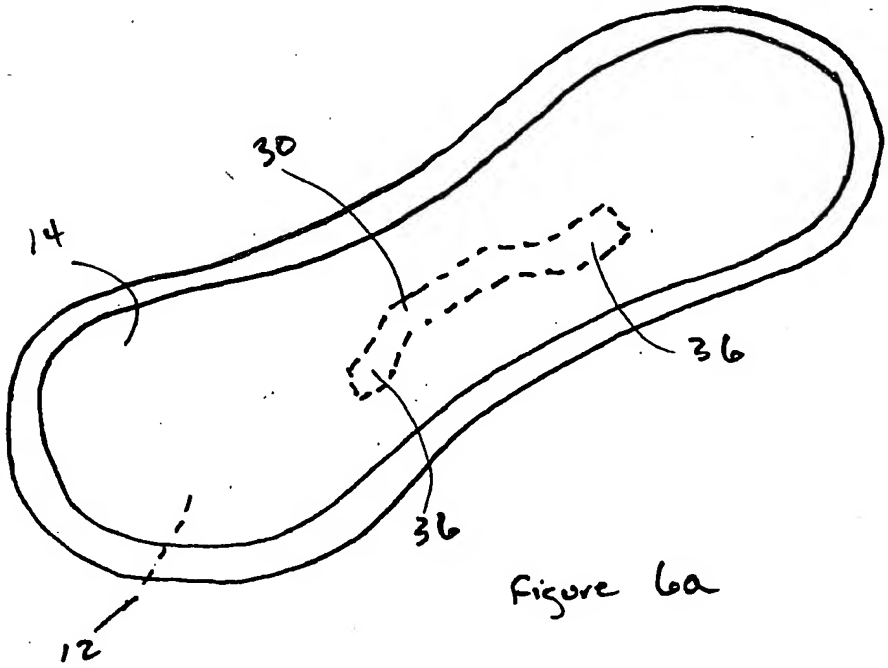


Figure 6a

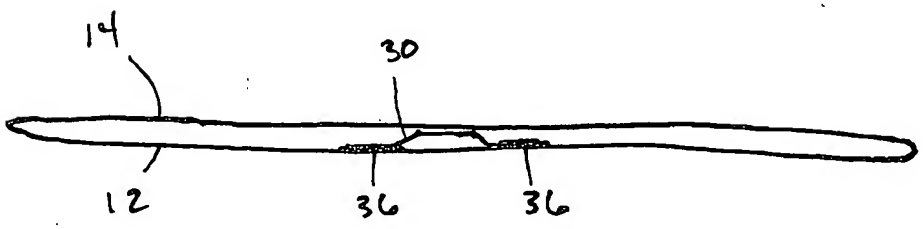


Figure 6b

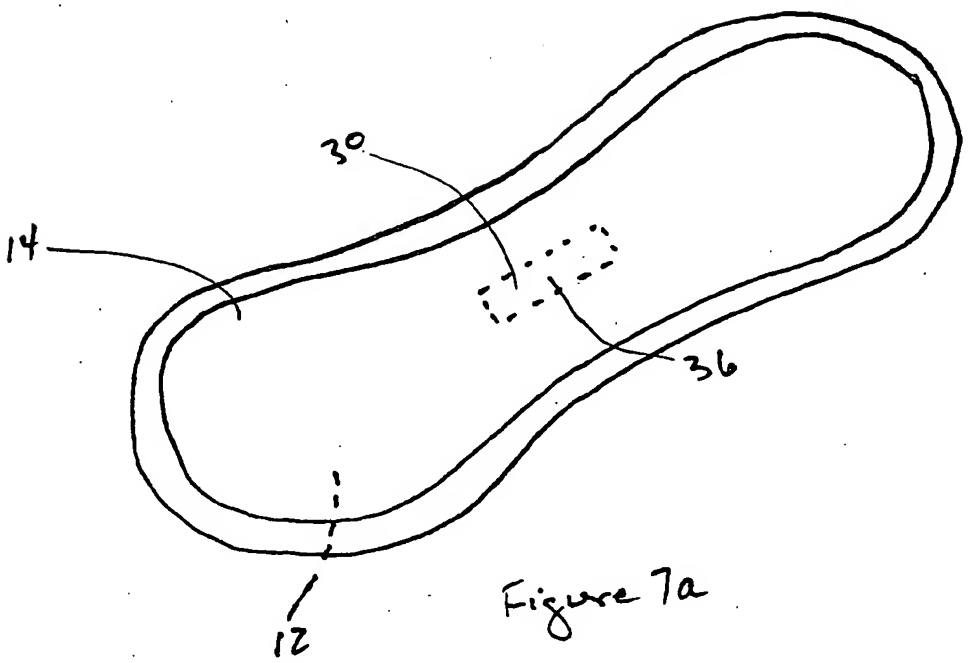
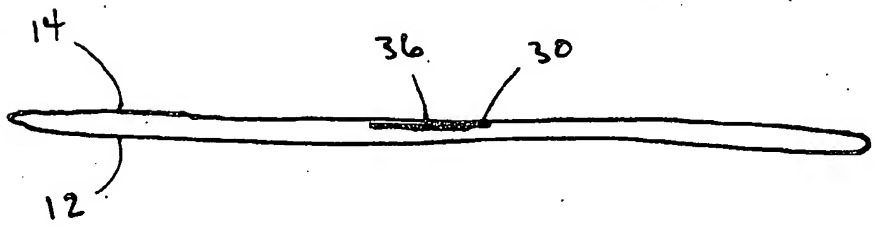


Figure 7a



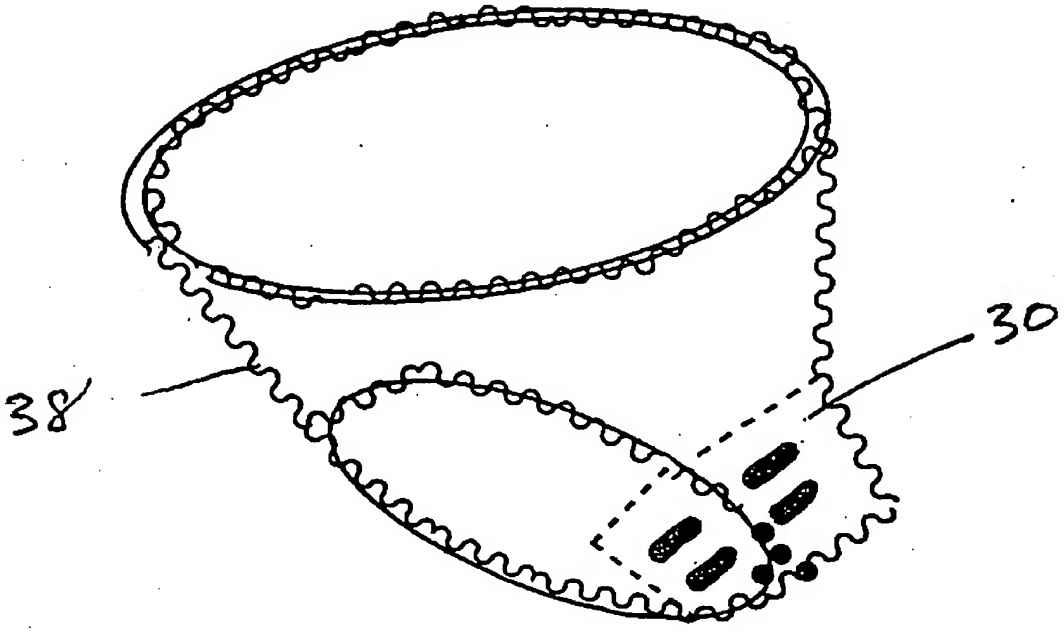


Figure 8

US 20050124072A1

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Boga et al. (43) Pub. Date: Jun. 9, 2005

(54) PERSONAL CARE PRODUCTS WITH VISUAL INDICATOR OF VAGINITIS

(75) Inventors: RameshBabu Boga, Roswell, GA (US); John Gavin MacDonald, Decatur, GA (US)

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(51) Int. Cl.[7] G01N 33/00

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(57) ABSTRACT

There is provided a personal care product having a body side liner, a baffle and an indicator strip with two ends. The indicator has an amine sensitive dye near at least one end. The indicator extends from the target area just below the liner to just above the baffle such that the dye deposit is visible to an unaided eye. The dye changes color in the presence of amines which are characteristic of infection, thus alerting the user to the possibility of infection. Such an indicator placed in a feminine hygiene pad, for example, may be useful in the diagnosis of vaginitis.

concentration of 50-5000 micrograms was placed on the strip on both ends in three relatively circular dots with a diameter of about 7 mm and allowed to air dry, producing an orange color. A strip (1.3 cmx8 cm) of the material having the three dots on each end was then inserted into the pad with the ends just above the baffle as shown in **FIG. 6**. The dots on the strip were visible through the baffle but the strip was not visible through the body side liner. The pad was then insulted with 200 microliters of vaginal secretion sample with spiked amines (1.5 mg/ml concentration of mixed amines TMA, 1,5-DAP, 1,4-DAB, and tyramine) placed in the target area of the pad. The orange color of the dye deposits changed color to a grey/black color after approximately<10 minutes, indicating the presence of amines and therefore serving as an alert signal for potential vaginitis. The dots of dye changed color sequentially with the dot closest to the target area changing color first and the others following in order.

EXAMPLE 6

[0061] A visual indicator was incorporated into a Kotex Lightdays Long (unscented) feminine hygiene pad as a small strip of nitrocellulosic material and demonstrated to be sensitive to the amine-based vaginal fluid. ANB dye at a concentration of 50-5000 micrograms was placed on the strip on both ends in three relatively circular dots with a diameter of about 7 mm and allowed to air dry, producing an orange color. A strip (1.3 cmx8 cm) of the material having the three dots on each end was then inserted into the pad with the ends just above the baffle as shown in **FIG. 6**. The dots on the strip were visible through the baffle but the strip was not visible through the body side liner. The pad was then insulted with 200 microliters of normal menses sample placed in the target area of the pad, and the solution was flowed from the center zone to the indicator zone by<5 minutes.

EXAMPLE 7

[0062] A visual indicator was incorporated into a Kotex Lightdays Long (unscented) feminine hygiene pad as a small strip of nitrocellulosic material and demonstrated to be sensitive to the amine-based vaginal fluid. ANB dye at a concentration of 50-5000 micrograms was placed on the strip on both ends in three relatively circular dots with a diameter of about 7 mm and allowed to air dry, producing an orange color. A strip (1.3 cmx8 cm) of the material having the three dots on each end was then inserted into the pad with the ends just above the baffle as shown in **FIG. 6**. The dots on the strip were visible through the baffle but the strip was not visible through the body side liner. The pad was then insulted with 200 microliters of menses sample with spiked amines (1.5 mg/ml concentration of mixed amines TMA, 1,5-DAP, 1,4-DAB, and tyramine) placed in the target area of the pad. The orange color of the dye deposits changed color to a grey/black color after approximately<10 minutes, indicating the presence of amines and therefore serving as an alert signal for potential vaginitis. The dots of dye changed color sequentially with the dot closest to the target area changing color first and the others following in order.

EXAMPLE 8

[0063] Assay devices were prepared from a nitrocellulose porous membrane (HF 12002 from Millipore, Inc.) having a length of approximately 30 centimeters laminated onto a corresponding supporting card (60 mmx301 mm from Millipore Corporation part number HF00MC100). Chemichromic detection zones were formed on each of the devices using a stock solution (~8.0 milligrams per milliliter) of alpha-naphtholbenzein (Sigma-Aldrich Chemical Company) in a methanol/water mixture (4/6 ratio). One (1) microliter of this stock solution was then spotted onto the assay devices to form the circular indicator zone, and the devices were then dried for 30 minutes at room temperature (25° C.). A cotton swab dipped normal vaginal sample was applied onto circular indicator zone to produce a color change from orange to grey/black color.

EXAMPLE 9

[0064] Assay devices as described above in Example 8 were used with a vaginal sample with additional "spiked" amines (1.5 mg/ml concentration of mixed amines TMA, 1,5-DAP, 1,4-DAB, and tyramine) and a cotton swab dipped in this solution was placed onto the circular indicator zone to produce a color change. The orange color of the indicator zone changed color to a grey/black color after 1-2 minutes, indicating the presence of amines and therefore serving as an alert signal for potential vaginitis.

EXAMPLE 10

[0065] Assay devices as described above in Example 8 were used for menstrual samples and cotton swabs dipped in the samples were placed onto the circular indicator zones to produce a color change.

EXAMPLE 11

[0066] Assay devices as described above in Example 8 were used for menstrual samples spiked with amines (1.5 mg/ml concentration of mixed amines TMA, 1,5-DAP, 1,4-DAB, and tyramine) and a dipped cotton swab was placed onto the circular indicator zones to produce a color change. The orange color of the indicator zone changed color to a grey/black color after 1-2 minutes, indicating the presence of amines and therefore serving as an alert signal for potential vaginitis. This experiment demonstrates the potential application for the visual indicator to detect amines from the center of the pad and act as visual alert to the user.

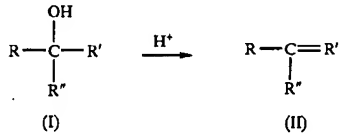
[0067] As will be appreciated by those skilled in the art, changes and variations to the invention are considered to be within the ability of those skilled in the art. Examples of such changes are contained in the patents identified above, each of which is incorporated herein by reference in its entirety to the extent it is consistent with this specification. Such changes and variations are intended by the inventors to be within the scope of the invention. It is also to be understood that the scope of the present invention is not to be interpreted as limited to the specific embodiments disclosed herein, but only in accordance with the appended claims when read in light of the foregoing disclosure.

What is claimed is:

1. A personal care product comprising an indicator having at least one deposit of an amine sensitive dye, placed in said product such that said dye deposit is visible to an unaided eye.

2. The personal care product of claim 1 wherein said dye is present in an amount of between about 0.0001 and 20 weight percent on a dry basis.

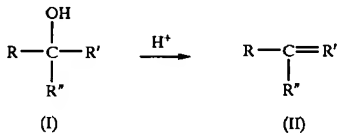
3. The personal care product of claim 1 wherein said dye is selected from the group consisting of chemicals of the general formula (I) or (II)



(I) (II)

where, R, R' and R" may each independently be a substituted aryl group, a napthyl group, heteroaryl groups and hydrogen.

4. The personal care product of claim 1 wherein said dye is selected from the group consisting of chemicals of the general formula (I) or (II)

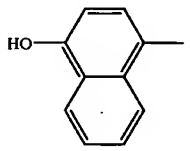


(I) (II)

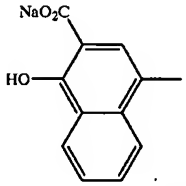
where,

R is H, $(NH_2)C_6H_5$- or C_6H_5-

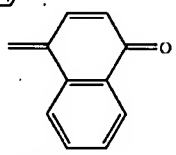
R' is $(CH_3)_2NC_6H$, $(NH_2)C_6H_5$-,



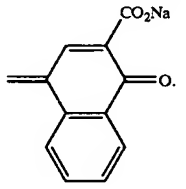
or



R" is $(CH_3)_2NC_6H_5$-, $(NH_2)C_6H_5$-



or



5. The personal care product of claim 1 wherein said dye is selected from the group consisting of pararosaniline base (PAB), alpha-naphthol-benzein (ANB), and naphthochrome green (NCG) and mixtures thereof.

6. The personal care product of claim 1 wherein said indicator is selected from the group consisting of cellulose, woven or nonwoven fabric, cotton, silk, rayon, glass fiber, films, silica gels and latex particles.

7. The personal care product of claim 6 wherein said indicator is between about 0.25 and 3 centimeters in width and about 8 to 25 cm in length.

8. The personal care product of claim 6 wherein said indicator is between about 1 to 2 cm in width and about 10 to 15 cm in length.

9. The personal care product of claim 1 wherein said personal care product is selected from the group consisting of and feminine hygiene pads and absorbent underpants.

10. A feminine hygiene pad comprising a liquid impervious baffle, a liquid pervious body side liner and having a target area, and an indicator having two ends and having at least one dried, solution applied deposit of an amine sensitive dye near an end, and wherein said indicator extends from immediately below said liner in said target area to immediately above said baffle.

11. The feminine hygiene pad of claim 10 wherein said amine sensitive dye is selected from the group consisting of pararosaniline base (PAB), alpha-naphthol-benzein (ANB), and naphthochrome green (NCG) and mixtures thereof and is placed in sequential dots near at least one end of said indicator.

12. The pad of claim 10 wherein said indicator is between about 1 to 2 cm in width and about 10 to 15 cm in length.

13. The pad of claim 10 wherein only one end of said indicator is placed adjacent said baffle.

14. The pad of claim 10 wherein both ends of said indicator are placed adjacent said baffle.

15. The personal care product of claim 1 wherein said personal care product is selected from the group consisting of and feminine tampons, swabs, removable patches, absorbent underpants.

16. A method of providing a system for visually indicating the presence of amines that are characteristic of vaginitis, the method comprising:

providing the feminine hygiene pad of claim 10,

providing instructions to enable a user to properly place the pad,

providing instructions to enable a user to visually examine the indicator at an appropriate time

providing instructions to enable a user to visually interpret changes in the indicator;

whereby a user is enabled to utilize the system to visually indicate the presence of amines that are characteristic of vaginitis.

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(54) ONE-STEP ENZYMATIC AND AMINE DETECTION TECHNIQUE

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(57) ABSTRACT

A technique for detecting the presence or quantity of an enzyme (or enzyme inhibitor) and/or an amine within a test sample is provided. For example, in one embodiment, a diagnostic test kit is employed that utilizes reactive complexes that each includes a substrate joined (e.g., covalently bonded, physically adsorbed, etc.) to a reporter and a separation species. Upon contacting the reactive complexes, enzymes may cleave the substrate and release the reporter. Moreover, the test kit may also employ a chemichromic dye, i.e., a dye that exhibits a detectable color change upon chemical reaction with one or more functional groups, such as amino groups. The signal generated (directly or indirectly) by the reporter and chemichromic dye may then be used to indicate the presence or quantity of an enzyme (or enzyme inhibitor) and amine, respectively, within the test sample.

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DOCUMENT-IDENTIFIER: US 20060003336 A1
TITLE: One-step enzymatic and amine detection technique

Description of Disclosure:
[0071] In some cases, triarylmethane dyes may be formed by converting a leuco base to a colorless carbinol and then treating the carbinol with an acid to oxidize the carbinol and form the dye. Thus, for example, pararosanilin may be derived by reacting the carbinol form of pararosanilin ("pararosaniline base") with an acid, such as, but not limited to, sulfonic acids, phosphoric acids, hydrochloric acid, and so forth. The carbinol form of pararosanilin is set forth below.

Description of Disclosure:
[0078] The chemichromic dye may be applied in a manner so that it does not substantially diffuse through the matrix of the chromatographic medium 23. This enables a user to readily detect the change in color that occurs upon reaction of the dye with an amine. For instance, the chemichromic dye may form an ionic and/or covalent bond with functional groups present on the surface of the chromatographic medium 23 so that it remains immobilized thereon. For example, in one embodiment, a positively-charged chemichromic dye may form an ionic bond with negatively-charged carboxyl groups present on the surface of some porous membranes (e.g., nitrocellulose). In other embodiments, the use of particles may facilitate the immobilization of the chemichromic dye at the amine detection zone 61. Namely, the dye may be coated onto particles, such as described above, which are then immobilized on the chromatographic medium 23. In this manner, the dye is able to readily contact a test sample flowing through the medium 23.

Description of Disclosure:
[0100] As indicated, the signal intensity exhibited by the first enzyme detection zone decreased in the presence of the enzyme, while the signal intensity exhibited by the second detection zone increased in the presence of the enzyme. The color of the amine detection zone changed from yellow to gray in the presence of the amine.

Description of Disclosure:
[0103] As indicated, the signal intensity exhibited by the first enzyme detection zone decreased in the presence of the enzyme, while the signal intensity exhibited by the second enzyme detection zone increased in the presence of the enzyme. The color of the amine detection zone changed from yellow to gray in the presence of the amine.

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TABLE 1

Qualitative Color Results for Detection Zones

Sample	First Enzyme Detection Zone	Second Enzyme Detection Zone	Amine Detection Zone
1	Weak	Strong	Gray
2	None	Strong	Yellow
3	Strong	None	Gray
4	Strong	Medium	Gray

[0100] As indicated, the signal intensity exhibited by the first enzyme detection zone decreased in the presence of the enzyme, while the signal intensity exhibited by the second detection zone increased in the presence of the enzyme. The color of the amine detection zone changed from yellow to gray in the presence of the amine.

EXAMPLE 5

[0101] The ability to detect the presence of an enzyme and amine in accordance with the present invention was demonstrated. Eight samples were tested. Each sample contained 25 microliters of the "BP-casein-B" of Example 1 (10 milligrams per milliliter); 600 microliters of Tween 20 (2%, Sigma-Aldrich Chemical Co., Inc.); and 900 microliters of Hepes buffer (pH of 7.2). Samples 1-8 contained cadaverine in an amount of 0.000 (amine control), 0.234, 0.468, 0.937, 1.875, 3.750, 7.500 and 15.000 micrograms per milliliter, respectively. Samples 1-8 also contained an active protease from *Bacillus polymyxa*, which is a metalloenzyme available from Sigma-Aldrich Chemical Co., Inc., in an amount of 0.0000 (enzyme control), 0.0275, 0.0550, 0.1100, 0.2200, 1.1000, 2.2000 and 11.0000 micrograms per milliliter, respectively. The samples were allowed to incubate for 10 minutes.

[0102] Each sample was then transferred to a well present on a microtiter plate. The assay device samples of Example 3 were then inserted into each respective well to initiate the test. After allowing the assay to develop for 10 minutes, the color intensity of each detection zone was observed. The qualitative results are set forth below in Table 2.

TABLE 2

Qualitative Color Results for Detection Zones

Sample	First Enzyme Detection Zone	Second Enzyme Detection Zone	Amine Detection Zone
1	Strong	None	Yellow
2	Strong	None	Yellow
3	Strong	Weak	Yellow/Gray
4	Strong	Medium	Yellow/Gray
5	Medium	Medium	Yellow/gray
6	Medium	Strong	Yellow/gray
7	Weak	Strong	Gray
8	None	Strong	Gray

[0103] As indicated, the signal intensity exhibited by the first enzyme detection zone decreased in the presence of the enzyme, while the signal intensity exhibited by the second enzyme detection zone increased in the presence of the enzyme. The color of the amine detection zone changed from yellow to gray in the presence of the amine.

EXAMPLE 6

[0104] The ability to detect the presence of an enzyme and amine in accordance with the present invention was demonstrated. Six samples were tested. Each sample contained 50 micrograms of the "BP-casein-B" of Example 1; Tween 20 (2%, Sigma-Aldrich Chemical Co., Inc.); and 40 microliters of tris buffer (pH of 7.4). Samples 1-6 contained cadaverine in an amount of 0.00 (amine control), 0.30, 0.60, 1.25, 2.50 and 10.0 milligrams per milliliter, respectively. Samples 1-6 also contained an active protease from *Bacillus polymyxa*, which is a metalloenzyme available from Sigma-Aldrich Chemical Co., Inc., in an amount of 0, 1, 2, 4, 8 and 40 nanograms per milliliter, respectively. The samples were allowed to incubate for 10 minutes.

[0105] Each sample was then transferred to a well present on a microtiter plate. The assay device samples of Example 2 were then inserted into each respective well to initiate the test. After allowing the assay to develop for 10 minutes, the reflectance intensity of each detection zone was measured using a reflectance reader. The quantitative results are set forth below in Table 3.

TABLE 3

Quantitative Color Intensity for Detection Zones

Sample	Reflectance Intensity (I_1) of First Detection Zone	Reflectance Intensity (I_2) of Second Detection Zone	Amine Detection Zone
1	2.0490	0.0263	0.2687
2	1.4070	0.6988	0.2890
3	1.1980	1.0920	0.2918
4	1.1080	1.3320	0.3169
5	0.8213	1.2800	0.3696
6	0.4298	1.2140	0.7020

[0106] As indicated, the signal intensity exhibited by the first enzyme detection zone decreased in the presence of the enzyme, while the signal intensity exhibited by the second enzyme detection zone increased in the presence of the enzyme. Also, the intensity of the amine detection zone increased in the presence of amine.

[0107] While the invention has been described in detail with respect to the specific embodiments thereof, it will be appreciated that those skilled in the art, upon attaining an understanding of the foregoing, may readily conceive of alterations to, variations of, and equivalents to these embodiments. Accordingly, the scope of the present invention should be assessed as that of the appended claims and any equivalents thereto.

1. A diagnostic kit for detecting an amine, enzyme, or an enzyme inhibitor within a test sample, the kit comprising:

a plurality of reactive complexes that each comprises a substrate joined to a reporter and a separation species, said substrate being cleavable by an enzyme to release said reporter; and

a chromatographic medium that defines a first enzyme detection zone within which an enzyme detection signal is capable of being generated, wherein the presence or quantity of an enzyme, or an inhibitor thereof, is determinable from said enzyme detection signal, said

chromatographic medium further defining an amine detection zone within which is contained a chemichromic dye, said chemichromic dye being capable of undergoing a color change in the presence of an amine, wherein the presence or quantity of an amine is determinable from said color change.

2. A diagnostic test kit as defined in claim 1, wherein the enzyme is a protease or peptidase.

3. A diagnostic test kit as defined in claim 1, wherein said substrate is a protein, glycoprotein, peptide, nucleic acid, carbohydrate, lipid, ester, or derivative thereof.

4. A diagnostic test kit as defined in claim 1, wherein said substrate is casein, albumin, hemoglobin, myoglobin, keratin, gelatin, insulin, proteoglycan, fibronectin, laminin, collagen, elastin, or a derivative thereof.

5. A diagnostic test kit as defined in claim 1, wherein said reporter comprises a detectable substance that is capable of directly generating said enzyme detection signal.

6. A diagnostic test kit as defined in claim 1, wherein said reporter comprises a specific binding member.

7. A diagnostic test kit as defined in claim 6, further comprising probes conjugated with a specific binding member, said probes comprising a detectable substance that is capable of directly generating said enzyme detection signal.

8. A diagnostic test kit as defined in claim 1, wherein said separation species is a specific binding member.

9. A diagnostic test kit as defined in claim 8, wherein a receptive material is immobilized within said first enzyme detection zone that has an affinity for said specific binding member.

10. A diagnostic test kit as defined in claim 1, wherein said separation species is a magnetic particle.

11. A diagnostic test kit as defined in claim 10, further comprising a magnetic device positioned adjacent to said chromatographic medium to immobilize said magnetic particle within a separation zone.

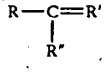
12. A diagnostic test kit as defined in claim 1, wherein said chromatographic medium further comprises a second enzyme detection zone within which a second enzyme detection signal is capable of being generated.

13. A diagnostic test kit as defined in claim 12, wherein a second receptive material is immobilized within said second detection zone that is capable of binding to said reporter or complexes thereof to generate said second enzyme detection signal.

14. A diagnostic test kit as defined in claim 12, wherein a second receptive material is immobilized within said second detection zone that is capable of binding to probes or complexes thereof to generate said second enzyme detection signal.

15. A diagnostic test kit as defined in claim 1, wherein said chemichromic dye is an arylmethane.

16. A diagnostic test kit as defined in claim 1, wherein said chemichromic dye is a triarylmethane having the following general structure:



wherein R, R', and R" are independently selected from substituted and unsubstituted aryl groups.

17. A diagnostic test kit as defined in claim 1, wherein said chemichromic dye is a diarylmethane.

18. A diagnostic test kit as defined in claim 1, wherein said amine detection zone is positioned downstream from said first enzyme detection zone.

19. A diagnostic kit for detecting an amine or a hydrolytic enzyme within a test sample, the kit comprising:

a plurality of reactive complexes that each comprises a substrate joined to a reporter and a specific binding member, said substrate being cleavable by a hydrolytic enzyme to release said reporter; and

a chromatographic medium that defines a first enzyme detection zone within which an enzyme detection signal is capable of being generated, wherein the presence or quantity of a hydrolytic enzyme is determinable from said enzyme detection signal, said chromatographic medium further defining an amine detection zone positioned downstream from said first enzyme detection zone, wherein a chemichromic dye is contained within said amine detection zone, said chemichromic dye being capable of undergoing a color change in the presence of an amine, wherein the presence or quantity of an amine is determinable from said color change.

20. A diagnostic test kit as defined in claim 19, wherein said reporter comprises a detectable substance that is capable of directly generating said enzyme detection signal.

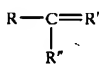
21. A diagnostic test kit as defined in claim 19, wherein said reporter comprises a specific binding member.

22. A diagnostic test kit as defined in claim 21, further comprising probes conjugated with a specific binding member, said probes comprising a detectable substance that is capable of directly generating said enzyme detection signal.

23. A diagnostic test kit as defined in claim 19, wherein said chromatographic medium further comprises a second enzyme detection zone within which a second enzyme detection signal is capable of being generated.

24. A diagnostic test kit as defined in claim 23, wherein said second detection zone is capable of capturing said reporter or complexes thereof to generate said second enzyme detection signal.

25. A diagnostic test kit as defined in claim 19, wherein said chemichromic dye is a triarylmethane having the following general structure:



wherein R, R', and R" are independently selected from substituted and unsubstituted aryl groups.

26. A diagnostic test kit as defined in claim 19, wherein said chemichromic dye is a diarylmethane.

27. A method for detecting an amine, enzyme, or enzyme inhibitor within a test sample, the method comprising:

i) contacting the test sample with a chromatographic medium, said chromatographic medium defining an enzyme detection zone and an amine detection zone, wherein an enzyme detection signal is capable of being generated within said enzyme detection zone and an

amine detection signal is capable of being generated within said amine detection zone;

ii) determining the presence or quantity of an enzyme or enzyme inhibitor from said enzyme detection signal; and

iii) determining the presence or quantity of an amine from said amine detection signal.

28. A method as defined in claim 27, wherein the quantity of an enzyme within the test sample is inversely proportional to the intensity of said enzyme detection signal.

29. A method as defined in claim 27, wherein the quantity of an enzyme within the test sample is directly proportional to the intensity of said enzyme detection signal.

30. A method as defined in claim 27, wherein said chromatographic medium further comprises a second enzyme detection zone within which a second enzyme detection signal is capable of being generated.

31. A method as defined in claim 30, wherein the quantity of an enzyme within the test sample is directly proportional to the intensity of said second enzyme detection signal.

32. A method as defined in claim 27, further comprising selectively controlling the pH level of the test sample to optimize the activity of an enzyme.

33. A method as defined in claim 27, wherein the test sample is obtained from vaginal fluid.

* * * * *